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Developmental puffing activity in the salivary gland and Malpighian tubule chromosomes of *Acricotopus lucidus* (Diptera, Chironomidae)

Wolfgang Staiber and Eckhard Behnke

Institut für Allgemeine Genetik,
Universität Hohenheim,
Garbenstrasse 30, D-7000 Stuttgart 70,
Federal Republic of Germany

Abstract

A detailed map of the salivary gland chromosomes of *Acricotopus lucidus* is presented. Differences in puffing and developmental puffing sequences of the three salivary gland lobes were investigated from mid fourth larval instar to pupation and compared with the puffing pattern of the Malpighian tubules. The intraglandular differentiation is quite extensive; the differences in the pattern of gene activity between the anterior lobe and the main and side lobes are as great as between the salivary gland and the Malpighian tubules. In the main and side lobes all developmental puffing changes proceed synchronously whereas in the anterior lobe both asynchronous and synchronous changes occur. In the anterior lobe the asynchronous regression of BR 3 and BR 4 is followed by a characteristic sequence of activation and inactivation of puffs.

Introduction

Gene activity can be visualized impressively in the puffing of polytene chromosomes. The observation of cell- and development-specific puffing activity in polytene chromosomes, as an expression of differential gene activity (Beermann 1952a, b; Mechelke 1953; Breuer and Pavan 1955), has been essential for an understanding of the fundamentals of cell differentiation. The dipteran *Acricotopus lucidus* (Chironomidae) exhibits striking cell- and development-specific differences in the puffing activity of the Balbiani rings in the anterior (AL), main (ML), and side

(SL) lobes of the larval salivary gland (Mechelke 1953, 1963, 1967). The electrophoretic pattern of the secretory proteins of AL and ML/SL is totally different (Baudisch 1977) and the amino acid hydroxyproline occurs specifically in the secretions of the ML and SL (Baudisch 1960). Ecdysone induces successive regression of the AL Balbiani rings BR 3 and BR 4 in the course of development to the prepupa (Panitz 1964; Panitz et al. 1972), in contrast to the unchanged activity of the ML/SL Balbiani rings BR 1 and BR 2. These observations, together with the specific development-dependent accumulation of carotenoids in the AL secretion (Mechelke 1953; Baudisch 1963), point to a far-ranging specialization of the gland lobes. Earlier cytological investigations of the salivary gland chromosomes of *A. lucidus* had been mainly concerned with the BRs and the nucleolus. The aim of the present investigation was to record the complete puffing patterns in the different gland lobes during normal larval development (mid fourth larval instar to pupation) and thereby to get a comprehensive survey of the genomic activity in the salivary gland and the degree of intraglandular differentiation at the chromosomal level. As a prerequisite for this, exact cytological chromosome maps were necessary. Only a few chromosome sections of *A. lucidus* had been published with a band designation (Mechelke 1960, 1961, 1963, 1967; Panitz 1964, 1965), therefore, as a basis for the puffing analysis, exact maps of the three salivary gland chromosomes of *A. lucidus* were drawn. The differentiation of a cell finds its expression in the synthesis of cell-specific proteins (Grossbach 1969). This correlates with the transcriptional activity of the gene loci which encode those proteins. The state of differentiation of a cell is reflected in the pattern of its genetic activity. Therefore an analysis of the puffing patterns and specific gene activity of another organ, the Malpighian tubules, was carried out in order to estimate how far the intraglandular differentiation of *A. lucidus* has progressed at the chromosomal level.

Materials and methods

Inbred lines of *Acricotopus lucidus* Staeger derived from aninals collected around Stuttgart-Hohenheim with chromosome I constitution CF (Wobus et al. 1971) were used. The larvae were reared at 10° to 12°C in glass vessels in daylight and were fed with *Spirogyra*. Panitz (1964, 1972) has given some information on the timing of larval development at this temperature (10°C): 1st larval instar ca. 320 h, 2nd larval instar ca. 215 h, 3rd larval instar ca. 225 h, 4th larval and prepupal stage until pupation ca. 435 h, pupal stage ca. 75 h. The changes in puffing pattern were investigated from mid-fourth larval instar (about 60 h after the last larval moult) to pupation (Fig. 1). The durations of the developmental stages determined by Panitz were averaged from different animals. An individual larva may develop either much faster or much slower, therefore, we classified the developmental stages not by time, as with other species, but rather with reference to the developmental-dependent regression of the AL BRs 3 and 4. These BRs regress in the same manner in both glands of an animal under the influence of ecdysone during transition from fourth larval instar to prepupa (Panitz 1964; Panitz et al. 1972). The BR regression begins firstly in the cells of the tip of the AL and proceeds successively in a gradient to the base of the AL (during a

period of 250 h at 10°C; Mechelke 1953; Panitz 1964). The BRs in the tip cells of the AL are the first sites in the salivary gland chromosomes to react visibly to the increasing titre of the moulting hormone. Therefore the present classification is closely related to the internal physiological state of the individual larva: 4.LS, 4th larval stage, BR 3 and BR 4 are expanded in all AL cells; 4.LS/PP,

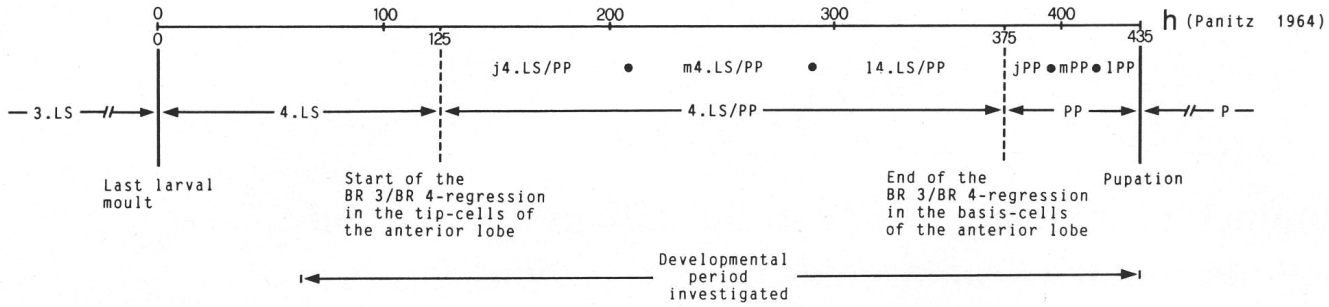


Fig. 1. Development of *Acricotopus lucidus* from the last larval moult to pupation at 10°C

transitional stage, in the AL, cells with expanded as well as cells with regressed BR 3 and BR 4 are present; PP, prepupal stage, BR 3 and BR 4 have regressed in all AL cells. For a more precise temporal classification 4.LS/PP and PP were further subdivided: j4.LS/PP, m4.LS/PP, 14.LS/PP and jPP, mPP, lPP, = young, middle, late transitional or prepupal stage (Fig. 1).

Salivary glands were explanted in haemolymph, fixed in ethanol/acetic acid (3/1, v/v; 2 h), stained with carmine-acetic acid (12 h) and then with orcein-acetic acid-lactic acid (1.5 h) and were then squashed in a drop of the latter. Malpighian tubules were dissected in Cannon's modified insect medium (Ringborg et al. 1970), fixed, stained and squashed as for the glands. For staining with light green, the method of Clever (1961) was followed, for methyl green- pyronin, the method of Kress (1972) and for toluidine blue, the method of Pelling (1964).

For each labelling experiment ten salivary glands were incubated in a mixture of 10 µl Cannon's modified insect medium, twofold concentrated, and 10 µl (5,6-^{3}H)-uridine (40-60 Ci mmol; 1 mCi/ml, Amersham, Buchler Braunschweig), at 11°C for 75-90 min. Squash preparations were covered with Kodak AR 10 stripping film, exposed for 12-33 days at 7 °C and developed in Kodak D 19.

Chromosome maps were drawn using a Neofluar 100/1,3 (Zeiss). Photomicrographs were made with a Zeiss photomicroscope II equipped with Planapo 63/1,4 and Planapo 100/1,3 optics.

Results

Salivary gland

The larval salivary gland of *Acricotopus lucidus* is morphologically subdivided into three distinct lobes, the anterior (AL), main (ML) and side (SL) lobes (Mechelke 1953; Fig. 2a, b). The lobes are arranged differently in each of the two glands of a larva, therefore Speiser (1973) has distinguished type 1 (Fig. 2a) and type 2 (Fig. 2b) glands. In the inbred lines used, the AL, with the expression of

BR 3, BR 4 and BR 7 and the ML and SL, with the expression of BR I and BR 2, exhibit a specific BR pattern (Mechelke 1953). In a gland, 12 to 16 cells show the AL-specific and 60 to 70 cells

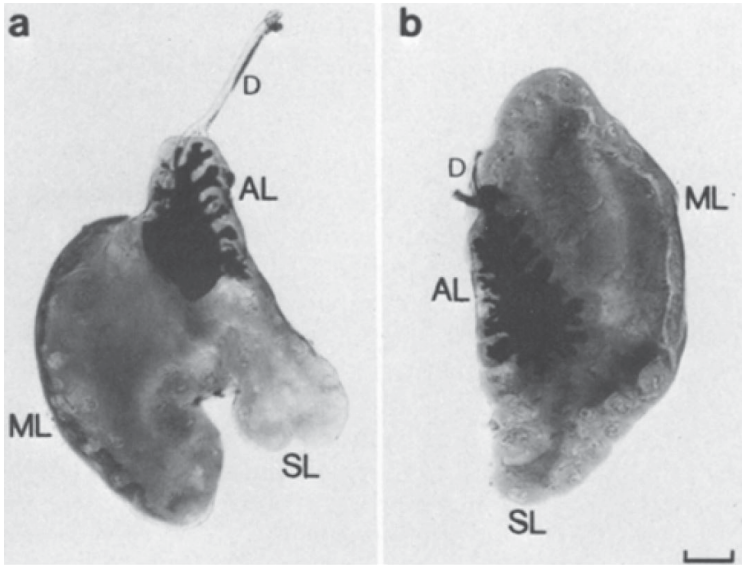


Fig. 2a, b. Salivary glands of *Acricotopus lucidus*, 4th larval instar. **a** Gland type 1. **b** Gland type 2. The specific staining of the anterior lobe secretion by paraldehyde-fuchsin shows the presence of acidic glycoproteins. **a** and **b** are from different animals. AL anterior, ML main, and SL side lobes; D glandular ductus. Bar represents 100 µm

show the ML/SL-specific BR pattern. Specific BR differences between ML and SL, for example an additional BR 6 in the SL as found in wild populations (Mechelke 1963, 1967), or other puffing differences, are not present. The ML and SL puffing patterns of the inbred lines investigated are identical. Therefore, all further data on the puffing in the ML also apply to the SL. Within a gland the nuclei do not all reach the same level of polyteny. In 4.LS it ranges from 1,024 to 4,096 C and in 4.LS/PP and PP from 2,048 to 8,192 C (Speiser 1973).

Fundamental differences in the composition of AL and ML/SL secretions have been detected by biochemical investigations (summary in Baudisch 1977). Another difference can be visualized using paraldehyde-fuchsin which stains acidic glycoproteins (Gabe 1976; Kolesnikov et al. 1981). As seen in Figure 2a, b the AL secretion contains such glycoproteins but the ML/SL-secretion does not. Sometimes the extruded AL secretion can be recognized as a violet ribbon in the common glandular ductus. In parallel with the successive ecdysone-induced regression of BR 3 and BR 4 and the accumulation of carotinoids in the AL secretion (Baudisch 1963), a change in the cell surface bordering on the gland lumen occurs. AL cells with expanded BR 3 and BR 4 have a very large surface area, characterisic of secretory activity, while in AL cells with regressed BR this is much reduced. In PP, when BRs 3 and 4 have regressed, the AL seems to be inactive in secretion. The EM studies of Döbel (1968) also support this observation.

Chromosomal maps and band nomenclature

Each of the three salivary gland chromosomes of *A. lucidus* exhibits a heterochromatic segment at a

characteristic position. These heterochromatin blocks located in a submedian (chromosome I), or nearly median (chromosomes II and III) position represent the centromeric regions and so each chromosome can be subdivided into a shorter left-hand and a longer right-hand arm (Mechelke 1953 ; Table 1).

Table 1. Parameters of the chromosome maps in Plate I

	I			II			III			Chromosome set
	I/1	C	I/2	II/3	C	II/4	III/5	C	III/6 +5A3/5A5	
Length of the unstretched chromosome arms and the centromeric regions at 2,048–4,096 C (µm) (Speiser 1972 and own results)	62	5 186	119	93	5 215	117	93	7 205	105	589 17 606
Length of the stretched chromosome arms and the centromeric regions in Plate I (µm)	114	5	219	171	5	215	171	7	193	1,083 17 1,100
Number of bands	224		420	345		459	363		405	2,216
Number of sections	5 (1A–1E)		9 (2A–2I)	7 (3A–3G)		10 (4A–4J)	8 (5–5H)		9 (6A–6I)	48
Active loci identified through band decondensation	30		56	52		65	51		51	305

The centromeric blocks, which are easy to recognize, provide clear reference points for chromosome mapping. The following nomenclature has been established for the salivary gland chromosomes of *A. lucidus* :

1.The chromosome arms IL/IR, IIL/IIR, IIIL/IIIR are numbered 1 to 6, so that left-hand arms receive odd, and right-hand arms even, numbers (IL = 1, IR = 2 and so on).

2. Starting from the centromere, each arm is subdivided into sections of 50 bands. The sections are marked alphabetically with capital letters.

3. Within each of these sections the bands are numbered consecutively, in the distal direction, from 1 to 99 in *odd numbers*, *even numbers* are assigned to the *interbands*. With this system each band and interband can be precisely and individually designated by a specific sequence of number, capital letter, number (for example 1B29, 4E55 or 2A94, 6F8). It is not possible to tell with certainty the number of bands composing the compact centromeric regions. The centre of the centromeric blocks is designated AO. The outer bands of the centromeres are designated 1A1, 2AI, 3A1 etc..

The results of the mapping are presented in Plates I and II. The drawings of the chromosome arms are based on the lengths summarized in Table 1. All three chromosomes, at a polyteny level of 2,048-4,096 C, have a length of 606 µm, unextended. In Plate I the arms (not the centromeres) are drawn extended and here the total length of the chromosome set is 1,100 µm. The total number of bands registered in the chromosome complement is 2,216 (Table 1).

Puff nomenclature

In order that each puff can be defined precisely, the designation of the puffs is based on the exact nomenclature of the bands. Following the number of the chromosome arm, those band(s) to which the initial locus (or the loci) of a puff can be delimited are put in parentheses, for example I(D 37), 6(B 7,9). All puffs which can be localized on the basis of band decondensation are represented in Plate I. The system is the same as that used by Pelling (1964) for *Chironomus tentans*. In AL and ML/SL, during the developmental period from mid-fourth larval instar to pupation, a total of 305 active loci can be identified by band decon- densation (nucleolus, 5 BRs and 299 puffs).

Classification of puffing activity, lobe-specific puffs, and characteristic differences in puffing activity of lobes

In the analysis of changes in puffing pattern the following classification of puff size class was used:

1. The band(s) are slightly decondensed or diffuse but can still be recognized as such [e.g. 4(D 91,93) in Figure 9b].
2. The band(s) are more decondensed. The puff is only slightly swollen [e.g. I(B 77, 79) in Figure 5e].
3. The puff is conspicuously swollen and its interior is completely diffuse [e.g. I(B 33-37) in Figure 5e].
4. Balbiani ring (e.g. BR 1 in Fig. 5e).

Some puffs only reach class 1 in activity and can only be observed sporadically. Therefore the behaviour of only 249 loci (out of 305 loci in Plate I) was recorded during mid 4.LS to pupation for AL and ML/SL

It could be ascertained that 14 loci puff in AL but not in ML/SL (i.e. AL-specific gene activity; Table 2). On the other hand eight loci could be observed to puff in ML/SL but not in AL (i.e. ML/SL-specific gene activity; Table 2). In Plate I AL-specific activity is marked with the symbol * and the ML/SL-specific activity with the symbol ●. By light green, methyl green-pyronin and toluidine blue staining, and also autoradiography, the lobe specificity of these puffs was checked and confirmed.

Some loci are active in all lobes but show characteristic quantitative differences in puffing activity in the different lobes. In AL 25 puffs exhibit a higher activity than in ML/SL; 14 puffs exhibit a higher activity in ML/SL than in AL (Table 2). In some cases the difference can only be observed in a specific developmental period. Quantitative differences in puffing activity of the same locus in different lobes, ascertained by band decondensation, could be confirmed by differences in tritiated uridine incorporation rates.

Identification of active sites by specific staining and autoradiography resulted in the localization of 44 additional puffs in which no band decondensation had been observed. Thus, a total of 349 active loci could be identified in the salivary gland chromosomes of *A. lucidus*.

Table 2. Comparison of the puffing activity of the different salivary gland lobes

Puffing activity	Number of active loci	% of total (249; including BRs and N)
AL = ML/SL	188	75.5
AL > ML/SL	25	10.0
ML/SL > AL	14	5.6
AL-specific	14	5.6
ML/SL-specific	8	3.2

AL = ML/SL, similar puffing activity in the AL and in the ML/SL. *AL > ML/SL*, *ML/SL > AL*, higher or lower puffing activity, respectively, in the AL than in the ML/SL. *BRs* Balbiani rings; *N* nucleolus

AL-specific	Figure	ML/SL-specific	Figure
2(C 5–9)	6a	BR 1	5d, e
2(E 53–59)	15a	2(B 73)	6c
BR 3	7a	2(G 19, 21)	7e
2(G 37)	7a, b	3(C 85, 87)	15d
3(C 55)	15c	BR 2	18a
4(C 7–11)	8a	4(D 91, 93)	9b, c
4(E 25, 27)	10d, 11a, b	5(B 81–85)	13e
BR 4	10a	6(G 53)	14e
BR 7			
5(B 17–21)	13c		
5(B 87, 89)	13a, b		
5(E 81, 83)	12b		
5(E 91–97)	12a, b, c		
6(B 7, 9)	15g		

AL > ML/SL	Figure	ML/SL > AL	Figure
1(B 11–15)		1(B 33–37)	5, d, e, f
1(B 91, 93)	5a	2(C 97)	
1(D 17)	5a	3(C 89–95)	
1(D 37)	5b	3(E 67, 69)	
2(B 41–65)	6a	3(F 99–G 3)	
2(D 9, 11)		4(A 71–77)	
2(D 15, 17)		4(G 93, 95)	
2(D 23)		4(I 77, 79)	
2(E 37–43)		5(F 5, 7)	12d
2(F 45)	7a, b	5(G 33, 35)	16c
3(F 43)		6(B 19,21)	15h
3(F 47)		6(C 7–11)	15h
3(F 73, 75)		6(E 27)	
3(G 25)		6(F 79, 81)	
4(A 93, 95)	15e		
4(D 55, 57)			
4(F 47–55)	11b, c		
4(G 83, 85)	11a, b		
4(H 21)			
4(I 55, 57)			
5(D 35–39)	12c		
5(G 69–75)			
6(G 3)	14a, b		
6(G 43, 45)	14c		
6(G 95–99)	14d		

There is normally an absolute separation of cells with AL-specific or ML/SL-specific puffing patterns. Only very rarely, out of more than 1,000 larvae examined, could cells with an intermediate

AL/ML puffing pattern be observed, probably as a result of fusion of adjacent AL and ML cells (Staiber, unpublished).

Developmental changes in puffing pattern

In the course of larval development some puffs do not change their puffing activity, while others clearly do so. In the AL 96 (40%) and in the ML/SL 79 (34%) loci show significant changes in puffing activity from mid 4.LS to pupation. The most conspicuous change in AL, the regression of BRs 3 and 4, is induced by ecdysone (Panitz 1964; Panitz et al. 1972). The regression of both BRs, however, does not occur synchronously in all AL cells of a gland but starts in the tip of the AL at the beginning of the 4.LS/PP and descends to the AL base. So AL cells with active BR 3 and BR 4 (base cells), and others with BRs which have regressed (tip cells) can be observed at the same time. This asynchrony in the AL is not limited to the regression of BRs but includes activation and inactivation of some other puffs. The period of asynchrony of these puffs starts in j4.LS/PP in the tip cells shortly after regression of the BRs and continues until mPP in the base cells. Thus the regression of BRs is the first sign of a fundamental change in the whole puffing pattern of an AL cell.

Besides the asynchronous changes, other puffing changes can be observed which are proceeding in all the AL cells of a gland at the same time (i.e. synchronously). In contrast to the AL, all changes in puffing pattern progress synchronously in the ML/SL.

Puffs which change during development can be classified by their activity into different types (modified after Kress 1972):

1. Loci which are already active in 4.LS and which (a) are inactivated or (b) reduce their activity strongly in the course of further development.
2. Loci, which are activated temporarily and which are (a) already active or (b) not active before their activation.
3. Loci with an increasing activity and maximal puff size in the PP, which are (a) already active or (b) not active before their increase in activity. The course of activity of the largest and most prominent AL and ML/SL puffs (during the period mid-4.LS to pupation), as well as the assignment to individual types, is summarized schematically in Figures 3 a, b and 4.

Puffing changes in the main and side lobes

ML and SL cells always exhibit a uniform pattern of gene activity so that the puffing situation in an individual ML/SL cell is representative of the puffing pattern in all the cells of these two lobes. In chronological order the most prominent changes in ML/SL are (Fig. 4):

- The nucleolus, fully developed in 4.LS, regresses gradually in the course of 4.LS/PP; this is the most conspicuous change in an ML/SL cell during 4.LS/VP; in jPP the nucleolar substance has mostly disappeared and the 5A3/5A5/nucleolar organizer/centromere complex is condensed.

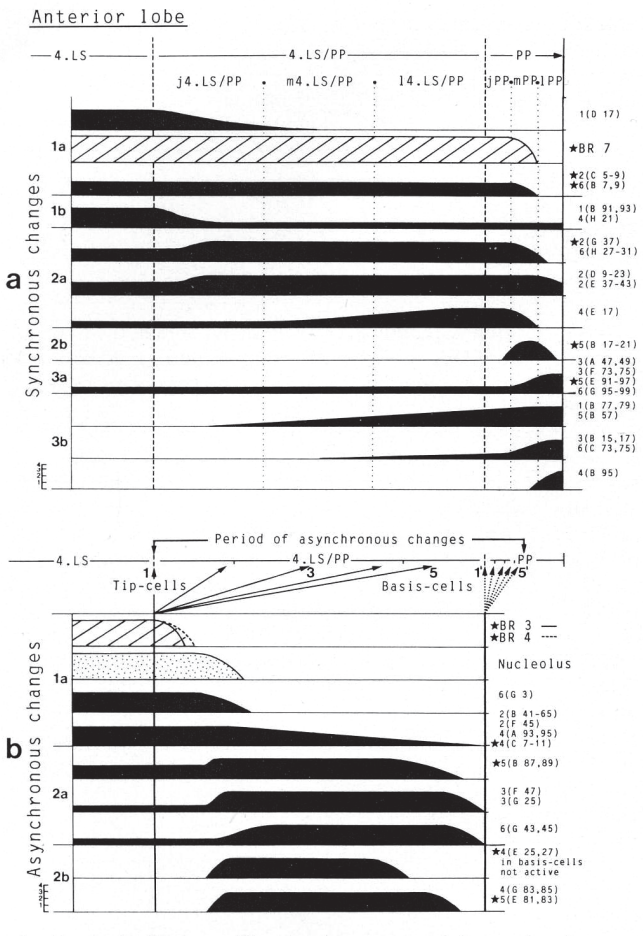


Fig. 3

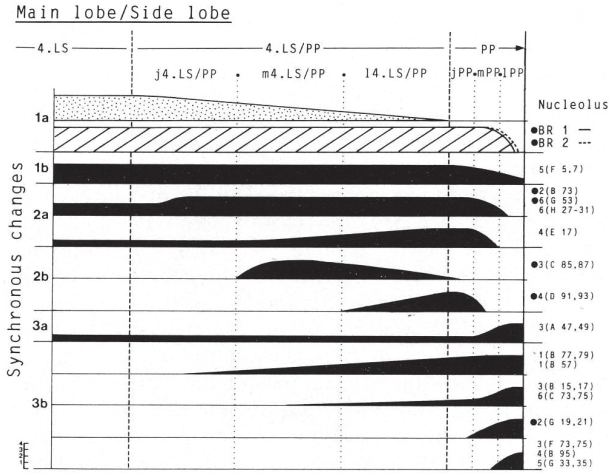


Fig. 4

Fig. 3a, b. Puffing profiles in the anterior lobe. **a** Synchronous puffing changes, **b** asynchronous puffing changes. The thickness of the lines represents the degree of puffing (see scale 1-4 on the left). The numbers la-3b indicate the reaction types (see text)

Fig. 4. Puffing profiles in the main and side lobes. All puffing changes proceed synchronously in these lobes

- The ML/SL-specific puffs 2(B 73) and 6(G 53) and the puff 6(H 27-31) intensify their activity in j4.LS/PP very clearly (to class 3); in mPP they reduce their activity and regress in lPP (Figs. 6c, d and 14e, f).
- In m4.LS/PP the ML/SL-specific puff 3(C 85, 87) develops and reaches class 3; in jPP it regresses completely (Fig. 15 d).
- Puff 4(E 17), only slightly active in 4.LS and j4.LS/PP, intensifies its activity in m4.LS/PP, reaches class 3 in 14.LS/PP and maintains this during jPP, until the end of mPP 4(E 17) regresses (Fig. 9a-d).
- The ML/SL-specific puff 4(D 91, 93) close to 4(E 17), is activated in 14.LS/VP; it reaches size class 3 in jPP and regresses in mPP, still before 4(E 17) (Fig. 9 a-d). The following puffs reach their maximal size in 1PP, just before pupation:
- Puff 3(A 47, 49), only slightly active during 4.LS and 4.LS/PP, intensifies its activity in mPP and reaches class 3 in 1PP.

- At 1(B 77, 79) and 5(B 57) the activation starts in j4.LS/P (Figs. 5d-f and 13e, f) and at 3(B 15, 17) and 6(C 73, 75) in m4.LS/PP to 14.LS/PP; all puffs reach class 3 in 1PP.
- In mPP the ML-specific puff 2(G 19, 21) (Fig. 7d, e) and the puffs 3(F 73, 75), 4(B 95) (Fig. 8c, d) and 5(G 33, 35) (Fig. 16c) are activated; in 1PP they reach class 3.

Sometimes in mPP and always in 1PP, a strong decrease in activity can be observed in BR 1 (Fig. 5f) and BR 2. Usually both BRs regress completely in 1PP just before pupation, with BR 1 always earlier than BR 2.

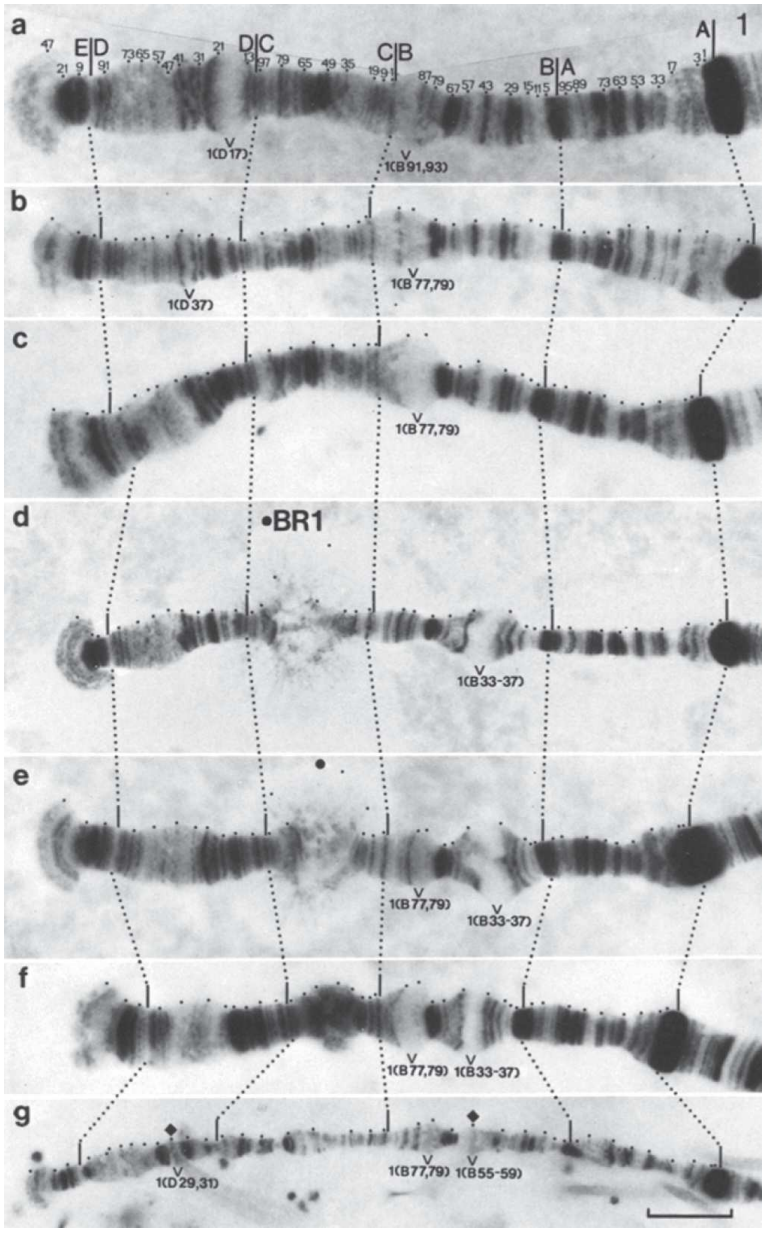
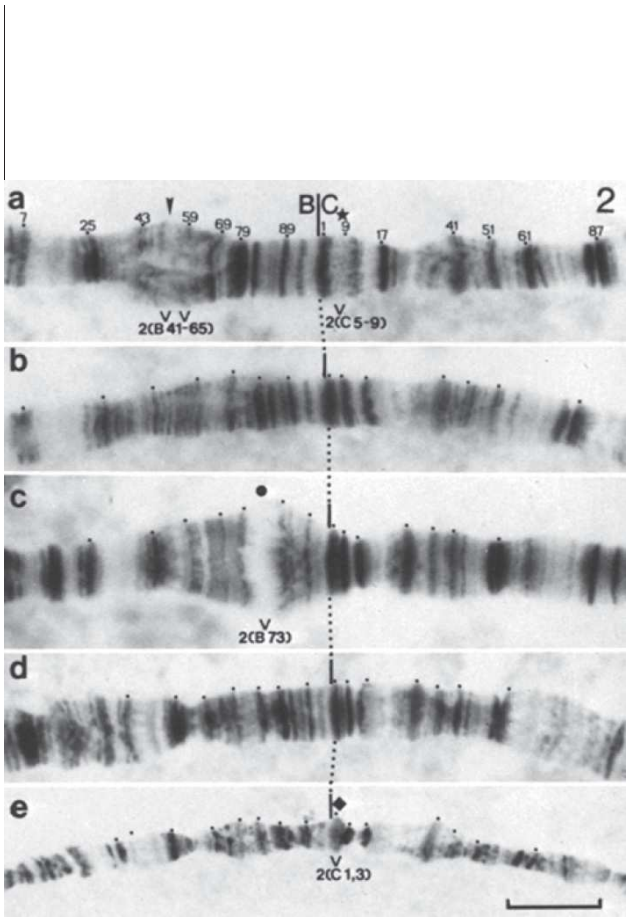


Fig. 5a-g. Development-dependent puffing changes and specific puffs in the left arm (1) of chromosome I. **a-e** Anterior lobe. **a** 4.LS, **b** 14.LS/PP, **c** 1PP. **d-f** Main lobe. **d** 4.LS, **e** 14.LS/PP, **f** 1PP. **g** Malpighian tubules, PP.

In the Figures anterior lobe specific activity is marked with the symbol *,main lobe specific activity with the symbol ● and Malpighian tubule specific activities with the symbol ♦. BR and puffs which change their activity asynchronously within the anterior lobe (with a tip to base gradient) are marked with the symbol▼. The number on the top of the right-hand side of the Figures indicates the chromosome arm in which the presented chromosome sections are located. If not otherwise stated, bars represent 10 µm



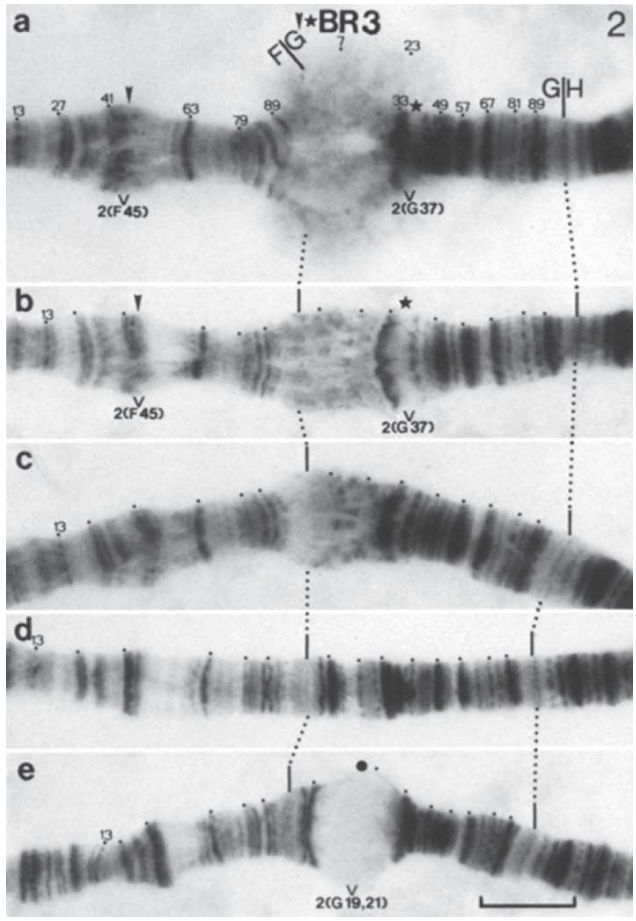


Fig. 6 **Fig. 7**

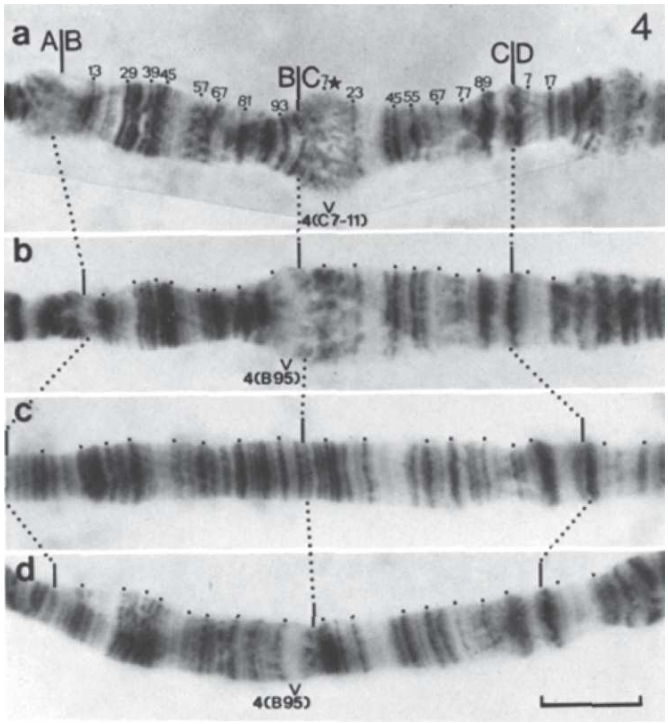
Fig. 6 a-e. Puffing changes and specific puffs in 2B/C. **a**, **b** Anterior lobe. **a** 4.LS, **b** 1PP. **c**, **d** Main lobe, **c** m4.LS/PP, **d** 1PP. **e** Malpighian tubules, PP

Fig. 7a-e. Puffing activity in the BR 3 region, 2F/G. **a**-**c** lobe. **a** Expanded BR 3 as found in 4.LS, and in 4.LS/PP but only in anterior lobe cells in the latter which have not reacted to ecdysone with asynchronous puffing changes, **b** Regressed BR 3, 4.LS/PP, **c** 1PP, **d**, **e** Main lobe. **d** m4.LS/PP, **e** 1PP

Puffing changes in the anterior lobe

The pattern of development-dependent puffing changes is more complex in AL than in ML/SL, because both synchronous and asynchronous changes occur in the former. In chronological order the most prominent synchronous changes in AL are (Fig. 3 a):

- Puff 1(D 17) exhibits mostly puff size class 2 or 3 in 4.LS and during j4.LS/PP and m4.LS/PP it regresses (Fig. 5a, b).
- The puffs 1(B 91, 93) (Fig. 5a-c) and 4(H 21) reduce their activity in j4.LS/PP from class 3 to class 1 but remain slightly active until pupation.
- The AL-specific puff 2(G 37) (Fig. 7a, b) and the puffs 2(D 9-23), 2(E 37-43) and 6(H 27-31) (Fig. 14a-d) are active in 4.LS and intensify their activity in j4.LS/PP up to class 3.
- Puff 4(E 17) shows the same behaviour as in ML/SL (Fig. 10 a-f).



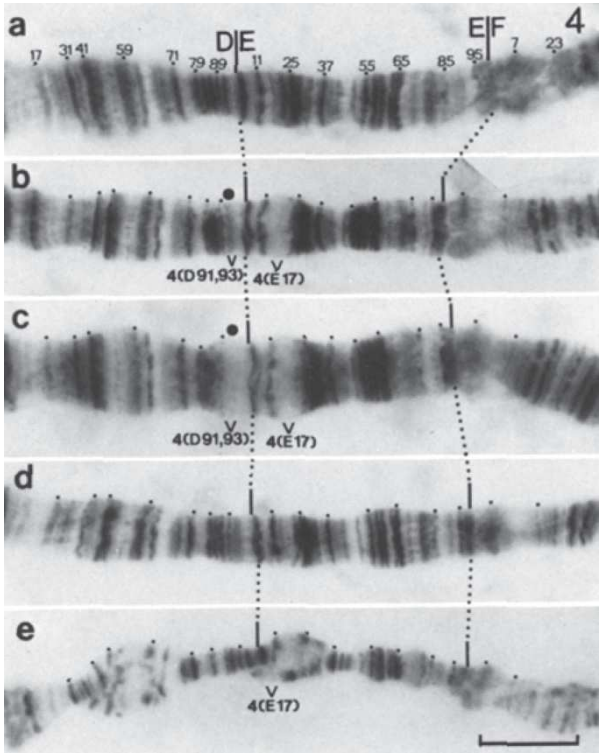


Fig. 8 **Fig. 9**

Fig. 8a-d. Puffing activity in 4B/C. **a**, **b** Anterior lobe. **a** Situation in 4.LS and in 4.LS/PP in anterior cells with expanded BR 4. **b** 1PP. **c**, **d** Main lobe. **c** Situation in 4.LS and 4.LS/PP. **d** 1PP

Fig. 9a-e. Developmental puffing sequence and specific puffs in the BR 4 region (4D/E) of the main lobe **a**-**d**. **a** 4.LS, **b** 14.LS/PP, **c** jPP, **d** 1PP. Malpighian tubules **e**, jPP

- The AL-specific puff 5(B 17-21) is activated in jPP, reaches class 3 in mPP and regresses in 1PP (Fig. 13 a-d).
- In mPP the AL-specific BR 7 and the AL-specific puffs 2(C 5 9) (Fig. 6a, b) and 6(B 7, 9) (Fig. 15 g) regress.

The following puffs reach their maximal size in 1PP just before pupation:

The AL-specific puff 5(E 91-97) (Fig. 12a-c) and the puffs 3(A 47, 49) (Fig. 16a), 3(F 73, 75) (Fig. 16b) and 6(G 95-99) (Fig. 14a-d) are only slightly active in 4.LS and 4.LS/PP but intensify their activity in mPP and reach class 3 in 1PP.

- The puffs 1(B 77, 79) (Fig. 5a-c), 5(B 57) (Fig. 13a-d), 3(B 15, 17) (Fig. 16a) and 6(C 73, 75) (Fig. 16d) show the same behaviour as in ML/SL.
- Puff 4(B 95) (Fig. 8 a, b) behaves similarly.

After BR 3/BR 4 regression a characteristic sequence of rapid changes in activity of certain loci occurs in an AL cell and these changes are also asynchronous within the AL. After the regression of the last BR 3 and BR 4 in the base cells and the ensuing sequence of inactivation and activation of puffs, the asynchrony of changes within the AL is not finished. It also extends to the regression of the puffs activated asynchronously. In the tip cells the inactivation of these puffs takes place in jPP,

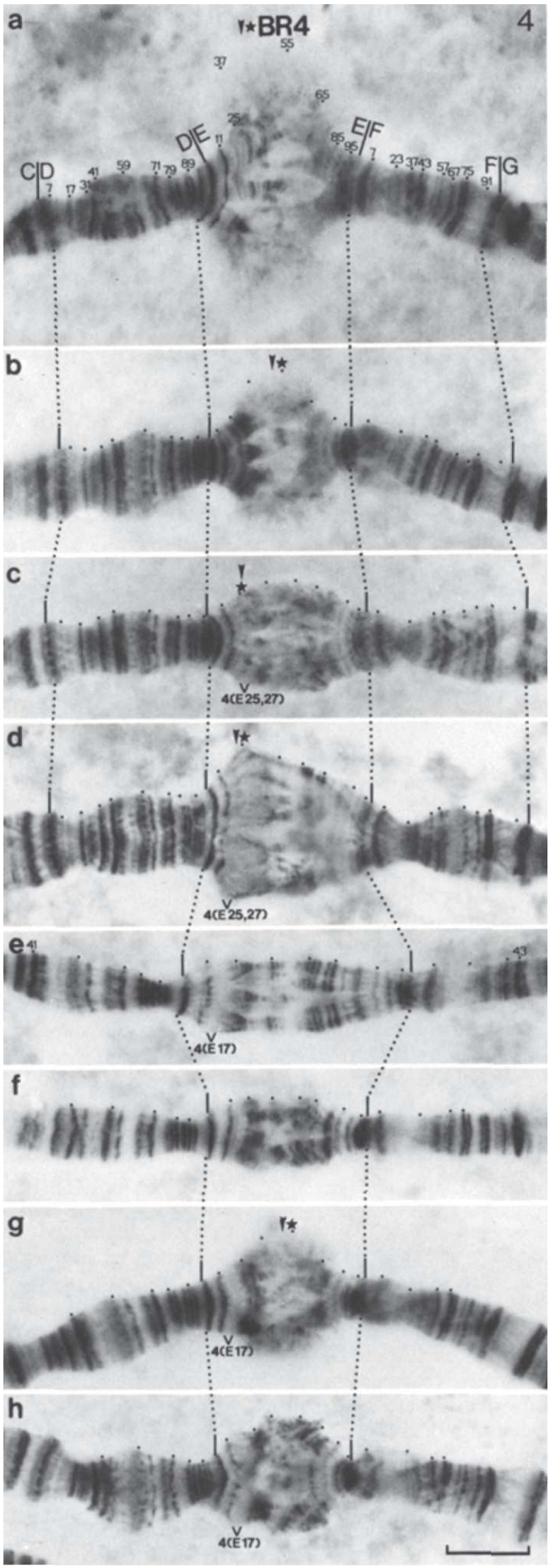

a
BR4
4
C|D
D|E
E|F
F|G
b
c
4(E25,27)
d
4(E25,27)
e
4(E17)
f
g
4(E17)
h
4(E17)

Fig. 10

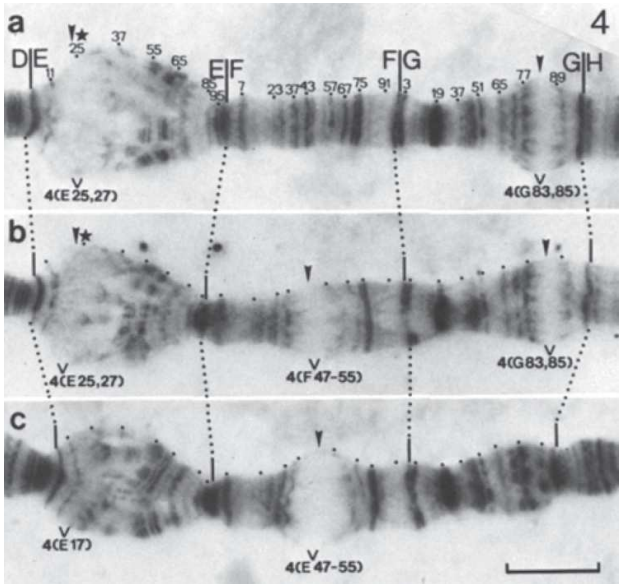

a
4
D|E
E|F
F|G
G|H
4(E25,27)
4(G83,85)
b
4(E25,27)
4(F47-55)
4(G83,85)
c
4(E17)
4(E47-55)

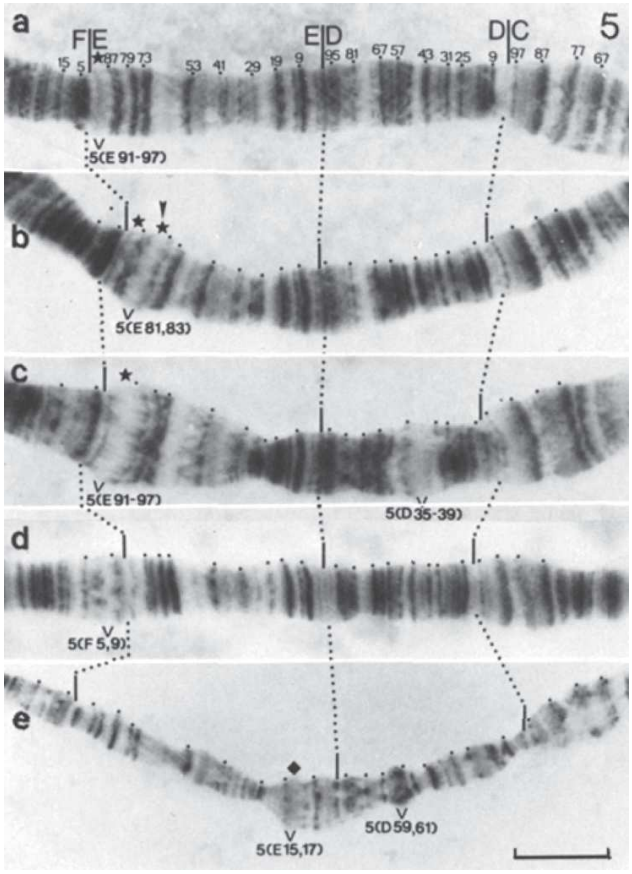
Fig. 11

Fig. l0a-h. Developmental changes in the BR 4 region of the anterior lobe. Migration of the puff maximum from distal to proximal along the chromosome. **a** Situation in 4.LS and in j4.LS/PP in anterior lobe cells which have not reacted to ecdysone. **b** BR 4 in regression in a tip cell of the anterior lobe, j4LS/PP. **c** BR4 has regressed, beginning of the activation of the AL-specific puff 4(E 25, 27), j4.LS/P. **d** Maximal activity of 4(E 25, 27), m4.LS/PP. **e** 4(E 25, 27) has regressed, maximal activity of 4(E 17), jPP. **f** 4(E 17) has regressed, 1PP. **g** and **h** demonstrate the parallel occurrence of synchronous and asynchronous puffing changes. **g** BR4 in regression as in **b** but here in a base cell of the anterior lobe in 14.LS/PP, therefore in contrast to **b** stage-specific activity of the synchronous puff 4(E 17). **h** jPP, base cell of the anterior lobe, BR 4 has regressed but activation of the AL-specific puff 4(E 25, 27) does not take place, maximal activity of the synchronous puff 4(E 17). BR 4 and 4(E 25, 27) change their activity asynchronously within the anterior lobe

Fig. 11a-c. Puffing in 4E/F/G, anterior lobe. **a**, **b** m4.LS/PP, **c** mPP. 4(G 83, 85) is only found when the BR 4 has completely regressed. 4(E 47-55) cannot be fixed to a definite developmental stage

in the base cells in mPP. Asynchrony in changes of puffing pattern therefore occurs in the AL in the period j4.LS/PP - mPP. The most prominent changes in the AL, which proceed asynchronously, are (Fig. 3 b):

- BR 3 and BR 4 regress completely, the regression proceeding faster in BR 3 than in BR 4 (Figs. 7a, b and 10a-c). The following changes occur shortly after BR 3/BR 4 regression in parallel in an AL cell. The nucleolus, which may be separated from the BR 7 but mostly forms a complex structure with BR 7 (Panitz 1972), regresses completely; BR 7 remains active. The regression occurs in a considerably shorter time than for the nucleolus in a ML/SL cell.
- Puff 6(G 3) regresses completely (Fig. 14a-c).
- The AL-specific puff 5(B 87, 89) (Fig. 13a, b) and the puffs 3(F 47) and 3(G 25), already active in 4.LS, intensify their activity clearly (to class 3).
- The AL-specific puff 5(E 81, 83) (Fig. 12b) and puff 4(G 83, 85) (Fig. 11 a, b) develop (both reach class 3).
- The AL-specific puff 4(E 25, 27) is activated and rapidly reaches class 3 (Fig. 10c, d). The activation of 4(E 25, 27), after the regression of BR 4 and after the delayed regression of 4(E 31-35) (located near the BR 4 locus, disrupted by the Balbiani ring and only visible in autoradiograms or in chromosomal mutations in which BR 4 and 4(E 31-35) have been separated), and its regression, together with the activation and regression of 4(E 17), gives the appearance of migration of the puff along the BR 4 region (Mechelke 1961 ; Fig. 10a-f). Puff 4(E 25, 27) is exceptional because it is formed in the tip cells (BR regression in j4.LS/PP) and in the cells of the middle part of AL (BR regression in m4.LS/PP) but not in the base cells (BR regression in 14.LS/VP; Fig. 10h).
- Shortly after the changes mentioned above, 6(G 43, 45) increases in activity. It reaches size class 3 but not before the regression of 6(G 3) is completely finished (Fig. 14a-c).
- After BR 3/BR 4 regression, one of the puffs in 2(B 41-65) (Fig. 6a, b), the two puffs 2(F 45) (Fig. 7a-c) and 4(A 93, 95) (Fig. 15e) and the AL-specific puff 4(C 7-1 I) (Fig. 8 a, b) reduce their activity gradually until they have completely regressed in mPP.



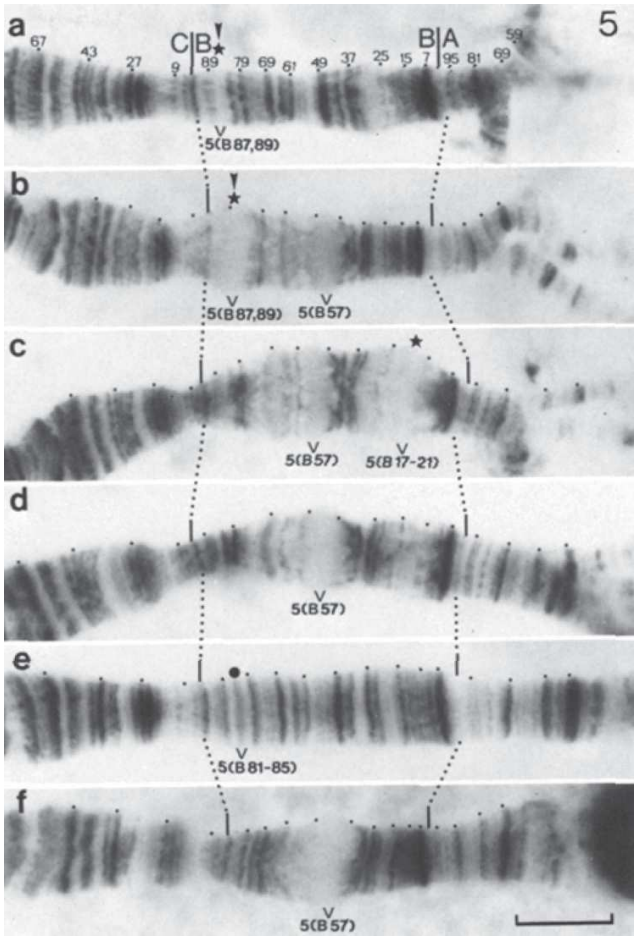


Fig. 12 **Fig. 13**

Fig. 12a-e. Puffing in 5D/E. **a**-**c** Anterior lobe. **a** Situation in 4.LS and 4.LS/PP when BR 3 and BR 4 have expanded. **b** Situation in 4.LS/PP when BR 3 and BR 4 have regressed, then the AL-specific puff 5(E 81, 83) is formed. **c** 1PP. **d** Situation in 4.LS and 4.LS/PP, main lobe. **e** PP, Malpighian tubules

Fig. 13a-f. Developmental puffing and specific puffs in 5B. **a**-**d** Anterior lobe. **a** Situation in 4.LS, j4.LS/PP and m4.LS/PP, when BR 3 and BR 4 have expanded,.**b** Situation in 4.LS/PP when BR 3 and BR 4 have regressed, **c** mPP. **d** 1PP. **a**-**c** The chromosomes are split in section 5A by BR 7. **e**, **f** Main lobe. **e** j4.LS/PP, **f** 1PP

One puff, 4(F 47-55) must be mentioned separately because its puffing behaviour cannot be clearly ascertained. It can be formed in m4.LS/PP (Fig. 11 b) and l4.LS/PP but also in jPP and mPP (Fig. 11 c). In m4.LS/PP and 14.LS/PP it can only be observed in cells with BRs 3 and 4 which have regressed. Therefore 4(F 47-55) changes its activity asynchronously. In ML/SL-cells, 4(F 47-55) exhibits only weak activity. The fact that in the AL during 4.LS/PP asynchronous and synchronous changes proceed side by side, can be demonstrated especially well with BR 4 and puff 4(E 17). In Figure 10b and 10g the BR 4 is just in regression but in contrast to Figure l0b (tip cell, j4.LS/PP) only in Figure 10g (base cell of an animal in 14.LS/PP) is the synchronous puff 4(E 17) fully developed. This is also apparent when comparing Figure 10c (j4.LS/PP) and Figure 10h (jPP). In both, the BR 4 has just regressed but only in Figure 10h is the puff 4(E 17) fully developed.

Due to the asynchrony of regression, BR 3 and BR 4 are active for a longer developmental period in the base cells than in the tip cells of the AL. The same applies to the nucleolus and 6(G 3). In

contrast, the phase of activity of the puffs activated asynchronously is much longer in the tip cells (from the beginning of j4.LS/PP to the end of 14.LS/PP, between 1 and 1' in Fig. 3 b) than in the base cells (from the beginning of jPP to mPP, between 5 and 5' in Fig. 3 b).

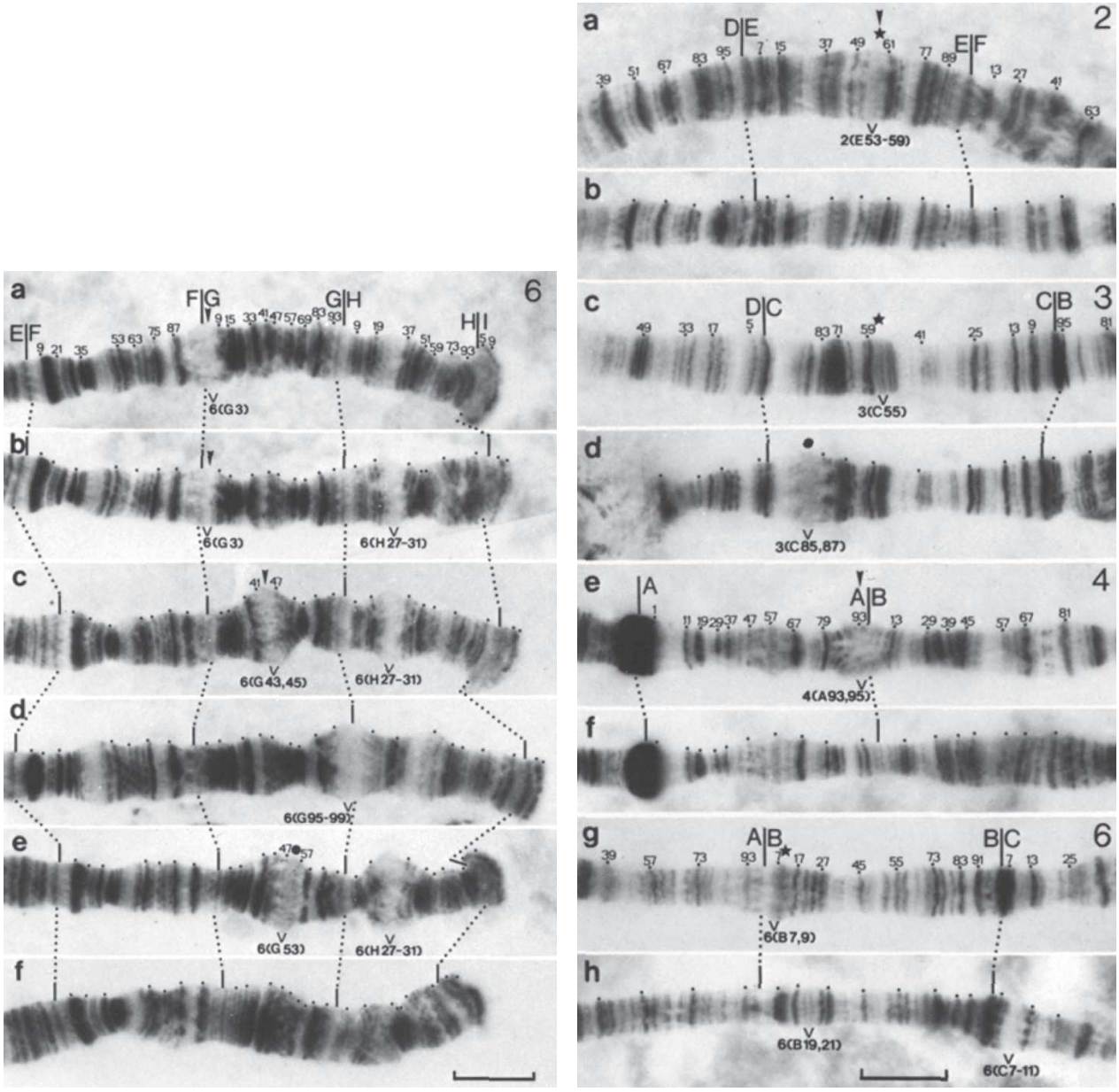


Fig. 14a-f. Developmental puffing and specific puffs in 6F/G/H/I. **a**-**d** Anterior lobe. **a** Situation in 4.LS and in 4.LS/PP in cells with expanded BR 3 and BR 4. **b**, **c** Puffing changes after BR 3 and BR 4 regression in 4.LS/PP and jPP. Regression of 6(G 3) and activation of 6(G 43, 45). **d** 1PP. **e, f** Main lobe. e 4.LS/PP. f 1PP

Fig. 15a-h. Anterior and main lobe specific puffs and puffs with lobe-characteristic quantitative differences in puffing activity, **a**, **c**, **e**, **g** Anterior lobe, **b**, **d**, **f**, **h** main lobe. **a** The AL-specific puff 2(E 53-59) is only formed after BR 3 regression

The chromosomes of the Malpighian tubules and their puffing activity

Malpighian tubules have excretory and osmoregulatory functions (Wessing and Eichelberg 1978). In contrast to the salivary glands they do not histolyse during metamorphosis. In *A. lucidus* each of the 4 unbranched tubules consists of 25-35 cells, which lie one behind the other in the tubule and

reach a polyteny level of 512-1,024 C in the prepupa (Speiser 1973), and 6-8 cells of very low polyteny. After the salivary glands, with 2,024-8,192 C, the Malpighian tubules have the second highest degree of polyteny of all tissues of *A. lucidus* (Speiser 1973). Their polyteny level is sufficient for an analysis of the puffing pattern.

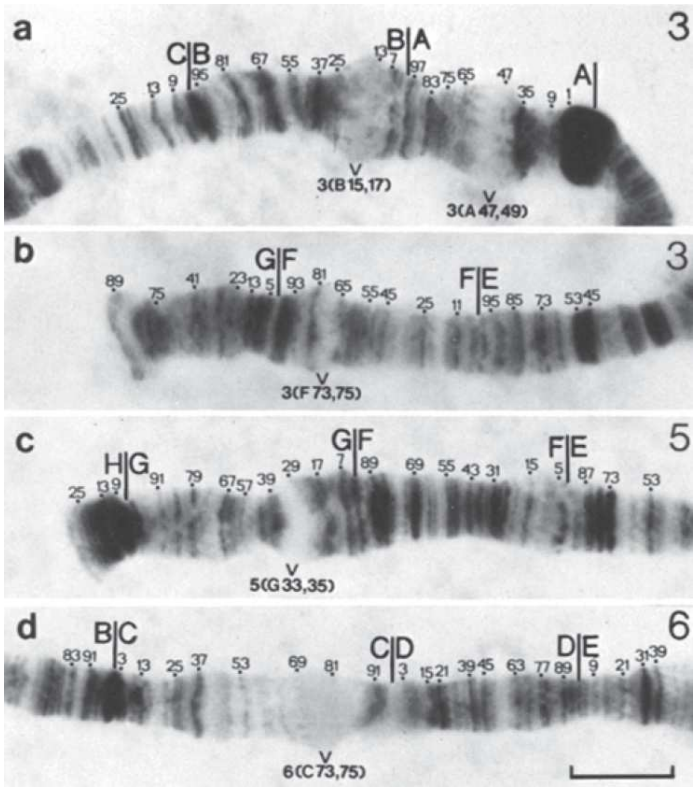


Fig. 16a-d. Puffs, which first reach their maximum size in 1PP, just before pupation, **a**, **b**, **d** Anterior lobe. **c** Main lobe

Because of their relatively low polyteny, Malpighian tubule chromosomes have a screw-shaped structure like those of the "Mäander-Typus" (Beermann 1952a, 1962). With a suitable squashing technique the chromosomes can be extended, causing the screw-shaped structure to disappear and the banding pattern to become clearer; smaller puffs can also then be identified better.

The banding pattern of the Malpighian tubule chromosomes of *A. lucidus* shows good homology with the banding pattern of the salivary gland chromosomes (for example Figs. 5 g, 6 e, 9 e). For a rapid identification of chromosomal sections characteristic darkly staining bands and easily remembered sequences can be used as landmarks. At a few places on the Malpighian tubule chromosomes, very faint bands were observed, which could not be discerned in the salivary gland chromosomes even though the latter were very much extended. The absolute band number of the genome of *A. lucidus* therefore exceeds 2,216.

The puffing pattern of the Malpighian tubules in 4.LS/PP and PP was analysed. A total of 204 active loci could be identified by band decondensation (nucleolus and 203 puffs). All puffs, excepting nine, were also active in the AL and ML/SL of the salivary gland. These Malpighian tubule specific puffs are listed in Table 3.

Table 3. Loci, which show specific puffing in the Malpighian tubules relative to the salivary gland

Locus	Figure	Locus	Figure
1(B 55–59)	5g	4(H 79, 81)	17c
1(D 29, 31)	5g	4(I 47)	17c
2(A 53, 55)	17a	5(E 15, 17)	12e
2(C 1, 3)	6e	5(F 71)	17d
2(I 39)	17b		

One of the puffs identified as AL-specific by comparing the puffs of AL and ML/SL (3(C 55), which reaches only puff size class 1 ; Fig. 15 c) was also active in the Malpighian tubules. Puff 3(C 55) is therefore not salivary gland specific.

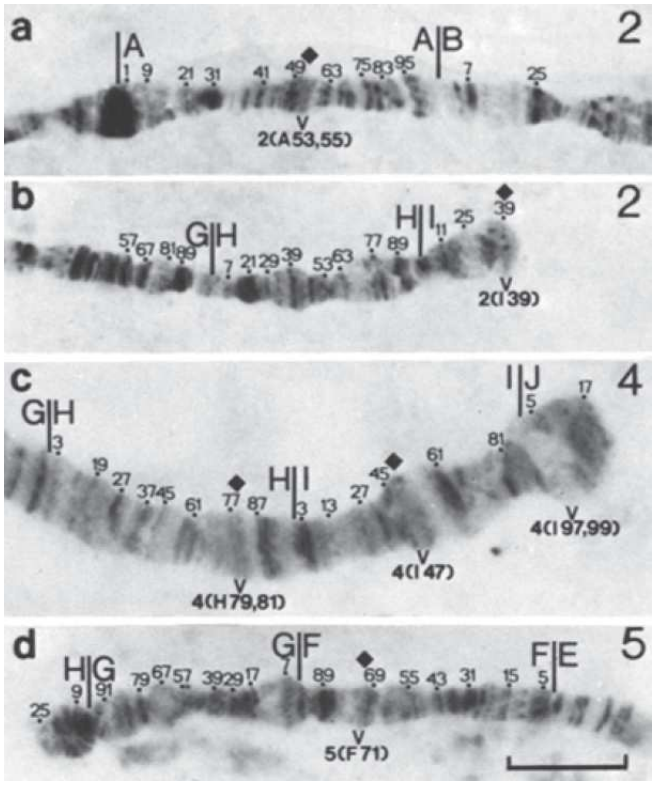


Fig. 17

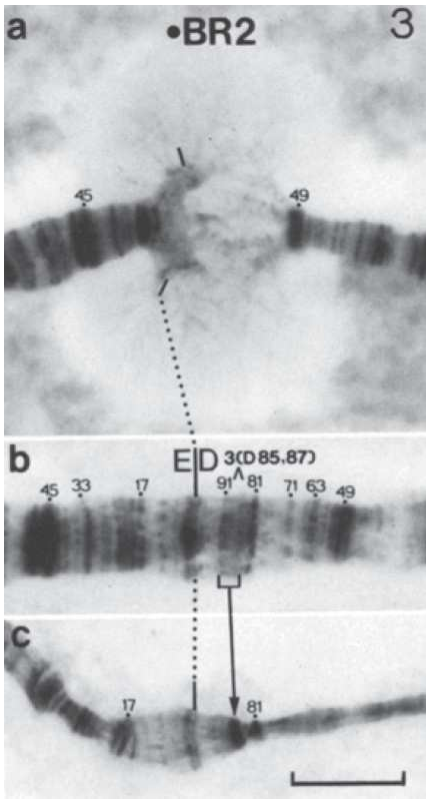


Fig. 18

Fig. 17a-d. Specific puffs of the Malpighian tubules **a-d.** The chromosome in **c** exhibits an especially high polyteny

Fig. 18a-c. BR 2 region in the main lobe **a**, in the anterior lobe **b** and in the Malpighian tubules **c**. The *arrow* in **c** indicates the compact condensed complex of bands in which the BR 2 locus is located

Also interesting is a comparison of the BR 2 region in the different gland lobes and the Malpighian tubules. While BR 2 is expanded in ML/SL (Fig. 18a) and in AL the puff 3(D 85, 87) has formed (Fig. 18 b), in the Malpighian tubules a strongly condensed complex of bands can be observed (arrow in Fig. 18 c).

Four puffs, 2(A 53, 55), a Malpighian tubule specific puff, 2(E 1) (Fig. 19a), 4(D 55, 57) (Fig. 19c), and 5(D 59, 61) (Fig. 12e), frequently exhibit an unusual appearance. In the regions of these

loci the chromosomes show unusual pairing and are slightly split there. In particular, 4(D 55, 57) sometimes exhibits a BR-like structure (Fig. 19 c). That each of these structures is caused by a puff becomes clearly visible when comparing Figure 19a and b. The pairing behaviour in these chromosomal regions is the same as observed by Richards (1980) in region 53D of the *Drosophila melanogaster* fat body chromosomes.

The most prominent change in the gene activity pattern during development in the Malpighian tubules is the regression of the nucleolus. In contrast to the regression of the nucleoli in AL (asynchronous, with a tip to base gradient) and in ML/SL (synchronous), which starts in j4.LS/PP about 125 h after the last larval moult (Fig. 1), the first signs of nucleolar regression in the Malpighian tubules can be observed in the transition from mPP to 1PP. This is only in a few cells, at about 400 h after the last larval moult. During the pupal stage nucleolar regression is completed and in the Malpighian tubules of adults, no nucleoli can be observed. In contrast, some Malpighian tubule puffs show developmental changes identical to those of the homologous puffs in the salivary gland, for example, 3(A 47, 49), 3(B 15, 17), 3(6 25), 4(g 17) (Fig. 9e) and 5(B 57).

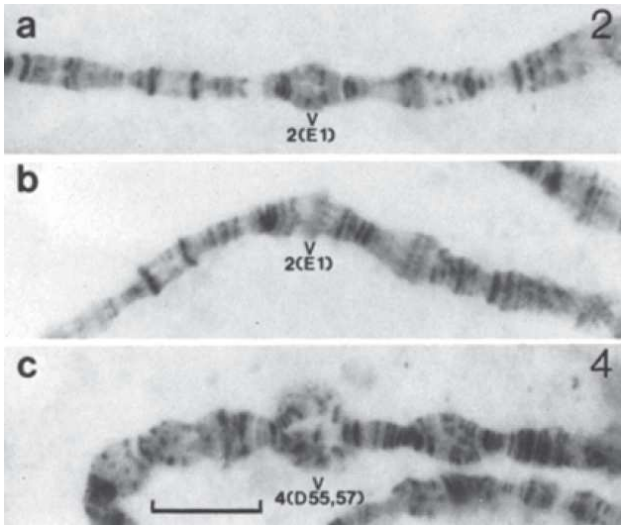


Fig. 19a-e. Regions of the Malpighian chromosomes with unusual pairing behaviour **a**, **c**. **b** The structure in **a** is caused by the puffing of 2(E 1). **c** The puff 4(D 55, 57) sometimes exhibits a BR-like structure

Because there is a great difference in polyteny, it is diffcult to compare the activity of puffs in the Malpighian tubule chromosomes with the same puffs in the salivary glands. Allowing for this, the following puffs exhibit significantly higher activity in the Malpighian tubules: 3(F 83, 85) (in the salivary glands this puff could only be identified by autoradiography and not by band decondensation), 4(E 71), 4(E 77, 79) and 4(I 97,99) (Fig. 17c).

Discussion

Nomenclature and band mapping

The new nomenclature for the chromosomes and bands of *A. lucidus*, established in the present investigation, takes the prominent, heterochromatic centromeric regions as reference points. Six chromosome arms can be defined easily. A thorough banding analysis, using very extended

chromosomes, led to a precise designation of the individual bands and interbands. Our puff nomenclature is adapted to single band analysis so that active loci lying directly side by side can be distinguished. Also, chromosomal rearrangements and breakpoints can be delimited and named exactly by this band/interband nomenclature. Each interband has a characteristic length as shown by EM investigations (Beermann 1972; Sorsa 1982).

With 2,216 bands, *A. lucidus* is within the range of the 2,000-5,000 bands Beermann (1972) has determined for Dipteran genomes and of the chironomids investigated, *A. lucidus* has the highest number of bands in the salivary gland chromosomes. Other examples are *Chironomus tentans*, 1,444 (Pelling 1964); *Chironomus thummi thummi*, 1,474 (Hägele 1970); ca. 1,600 after EM investigation Kiknadze et al. 1976); *Glyptotendipes barbipes* ca. 1,200 (Walter 1973); *Lundstroemia parthenogenetica* ca. 1,300 (Porter 1971) and *Prodiamesa olivacea* ca. 1,230 (Zacharias 1979). The band number of *A. lucidus* is more consistent with calculations made from polytene chromosomes of tissues other than the salivary glands, e.g. in *Chironomus tentans* with ca. 1,900 bands, the calculation of the band number of midgut chromosome III (Beermann 1952a) or the ca. 2,500 bands, by extrapolation from EM studies, of Malpighian tubule chromosome IV. Tusscher and Derksen (1982) found about 75% more bands in the latter than in salivary gland chromosme IV (salivary gland chromosome set, 1,444 bands; see above). It also fits within the range of the band numbers of most *Drosophila* species, for example *D. gibberosa* 2,019 bands (Roberts and MacPhail 1985), *D. hydei* 1,988 (Berendes 1963), *D. immigrans* ca. 2,000 (Le Calvez 1953) and *D. virilis* ca. 2,000 (Patterson et al. 1940). Only *D. melanogaster* is an exception with 5,059 bands (Leferve 1976).

Puffing activity

In *A. lucidus* 305 active loci have been identified by band decondensation in the salivary gland chromosome set. Special staining for puffs and ^{3}H-uridine autoradiography have extended this to 349. Thus, *A. lucidus* falls within the range that has been ascertained from the salivary glands of other Diptera studied intensively. The range includes *C. tentans* with 277 (Pelling 1964), *C. thummi* with 336 (Kiknadze 1981), *D. melanogaster* with 341 (Zhimulev 1974) or 355 (Vlassova et al. 1985) and *D. virilis* with 348 active loci (Kress 1972).

In the Malpighian tubules of *A. lucidus* only 204 active loci could be identified by band decondensation. The smaller number as compared with the salivary gland may be due to the lower polyteny of the Malpighian tubule chromosomes which made it more difficult to detect loci with only slight puffing activity. When comparing the puffing of Malpighian tubules and salivary glands, it is evident that even different tissues (with the exception of a few tissue- specific gene activities) can share a common set of active loci. Of the 204 active loci identified in the Malpighian tubules, 195 are in fact also active in the salivary glands. This may apply to most other tissues and is supported by studies of the puffing pattern of different polytene tissues of *D. hydei* (Berendes 1965b, 1966).

Comparative studies of the mRNA cytoplasmic sequences of different tissues of the same organism have demonstrated that the majority are common to all tissues, about 85%, for example, in chicken liver and oviduct (Axel et al. 1976; Hastie and Bishop 1976; Young et al. 1976). Comparing the nuclear RNA sequences of different tissues gives an even higher concordance. Chikaraishi et al. (1978) have found that, in the rat, at least 94% of the liver nuclear RNA sequences are present in the brain nuclear RNA, and at least 95% of the kidney nuclear RNA sequences are present in the liver nuclear RNA. This correspondence is qualitative not quantitative.

When determining the number of copies of the different poly-A-containing sequences present in a single cell, Bishop et al. (1974) established three abundance classes in HeLa cells:

1. mRNA sequences with ca. 10^4 copies/cell (" super prevalent class", Davidson and Britten 1979), 17 different sequences.
2. mRNA sequences with ca. 450 copies/cell ("moderately prevalent class", Davidson and Britten 1979), 370 different sequences.
3. mRNA sequences with only few copies/cell ("complex class", Davidson and Britten 1979), 33,000 different sequences.

The occurrence of these abundance classes, and the order of magnitude of the number of different mRNA sequences present in classes 1 and 2, has been confirmed by further investigations, e.g. with *Drosophila* cells and mouse and chicken tissues (Levi and McCarthy 1975; Hastie and Bishop 1976; Axel et al. 1976). The extraordinarily high number of 33,000 mRNA sequences in class 3 seems to be specific for HeLa cells; in the other investigations mentioned it ranged from 6,700 to 15,000.

In classes 1 and 2 together there are about 400 different mRNA sequences the genes of which most probably exhibit enhanced transcriptional activity. It is tempting to equate the 300-350 puffs visible in the salivary gland with these genes as only these visible puffs are the sites of intensive RNA synthesis (Pelling 1964). The high number of ca. 2,000 transcriptionally active loci in the salivary glands of *D. melanogaster* claimed by Ananiev and Barsky (1978), using chromosomes stretched by micromanipulation for autoradiography, may be the result of genes for class 3 mRNAs, with very low transcriptional activity, being detected.

The results of Rodgers and Shearn (1977) support the assumed relationship between the class 1 and 2 mRNA sequences and the cytologically visible puffs. These authors ascertained, by two-dimensional gel electrophoresis, that in different imaginal discs (wing, leg, eye-antenna) of larvae of *D. melanogaster* between 300 and 350 different polypeptides are always synthesized in greater amounts in a distinct period of time. This is in surprising agreement with the number of class 1 and 2 mRNA sequences and with the puff numbers mentioned above. Equally interesting is the fact that 81 to 85% of the polypeptides can be found in all three imaginal discs (Rodgers and Shearn 1977). This agrees with our observation that, in such different tissues as Malpighian tubules and salivary glands, the puffs present are nearly all the same.

Tissue-specific puffing activity

Differences in puffing patterns between polytene cells indicate functional differentiation and have already been observed several times within the salivary glands of Dipteran larvae. This has led to the differentiation of special cells from main cells, to the distinction of different lobes and to the division of the glands into proximal and distal parts. Examples include *Chironomus pallidivitatus* (Beermann 1961), *Chironomus thummi* (Kolesnikov et al. 1981), *Drosophila hydei* (Berendes 1965a), *Rhynchosciara holleanderi* (Stocker and Pavan 1977), *Sciara coprophila* and *Sciara pauciseta* (Gabrusewycz-Garcia 1971) and *Trichocladius vit- ripennis* (Beermann 1952b).

Often the differences in puffing are restricted to the occurrence of only one specific BR, as in *C. pallidivitatus* and *C. thummi*. In the salivary gland of *T. vitripennis*, with one specific BR in ordinary cells and two in the granular cells and in the salivary glands of *R. holleanderi*, *S. coprophila* and *S. pauciseta*, with the specific activity of several puffs and some quantitative puffing differences between the different gland regions, the intra-gland differentiation is already more advanced. With 14 AL- and 8 ML/SL-specific activities and many characteristic quantitative differences in activity, the functional differentiation within the salivary gland of *A. lucidus* is more advanced than in all other Dipteran species investigated so far.

Detailed puffing analyses of different polytene tissues of the same organism have been carried out on *D. hydei* (salivary gland, Malpighian tubules and midgut; Berendes 1965b, 1966), and on *R. americana* (salivary glands, Malpighian tubules and gut; Guevara and Basile 1973) and between two and ten tissue-specific puffs have been observed. The Malpighian tubules of *A. lucidus* have nine specific puffs when compared with the salivary glands.

The cell or tissue specificity of an active locus can only be established by comparing it with the activity in another cell type or tissue. The salivary gland BRs are absolutely specific for that gland because the mRNAs of the secretory proteins are transcribed there (Case and Daneholt 1978). The same seems to be true for the DNA puffs of the sciarids; for some, a direct relationship with the spinning proteins of the cocoon has been established (Winter et al. 1977). From the few data on the number of specific puffing activities in polytene tissues, one can infer that the differences in puffing between the AL and ML/SL of *A. lucidus* are of the same magnitude as those observed between different tissues.

Developmental puffing changes

Development-dependent changes in puffing patterns are mostly connected with the release of the moulting hormone ecdysone (Clever 1961 ; Becker 1962; Panitz 1964). In *Drosophila*, as in *Chironomus*, characteristic puffing sequences with the occurrence of early and late puffs have been observed (Becker 1959; Clever 1962; Ashburner 1972) and control models developed (Clever and Romball 1966; Ashburner et al. 1974).

In contrast to *D. melanogaster*, where development-dependent fluctuations have been found in the increase of the ecdysone titre in the haemolymph (Borst et al. 1974), the ecdysone titre in the chironomid *C. thummi* increases steadily from the end of 4.LS until pupation (Valentin et al. 1978).

Starting with the release of ecdysone, characteristic puffing sequences also occur in the AL and ML/SL of *A. lucidus*. Initially the AL tissue-specific loci - BR 3 and BR 4 - are inactivated, the earlier functions of the AL cells presumably being fulfilled. Inactivation of gene loci is also the first response in *Drosophila*. Here those puffs which code for the glue proteins are repressed (Korge 1975; Velissariou and Ashburner 1980). The production of the glue proteins seems to be the main function of the salivary gland of *Drosophila*. The regression of these intermoult puffs follows the formation of new puffs (early, mid-prepupal and late puffs) and the synthesis of a new saliva of unknown function (Korge 1977). Thus a change in salivary gland function takes place but whether the AL of *A. lucidus* changes its function, cannot be clearly answered. After the regression of the BRs some large puffs are formed (some AL-specific) and an accumulation of carotinoids in the AL secretion occurs although no new secretory proteins have been detected (Baudisch 1963, 1977)

In the ML/SL, in contrast to the AL, no regression of ML/SL-specific puffs occurs as the ecdysone titre increases and no change of function takes place. ML and SL thus behave like the salivary gland of *C. tentans* where no regression of a BR or puff is induced by ecdysone (Clever 1961). The sequence and nature of changes in puffing patterns in ML/SL are very similar to those Clever (1962) has observed in *C. tentans*. Clever has described:

1. Early puffs, for example 1-18-C and IV-2-B, which are not active or are only slightly so in 4.LS, and which, during normal development, are the first loci to be activated after the release of ecdysone. After injection of ecdysone they appear within 15-60 min. In *A. lucidus* no comparable injection experiments have been carried out but from their time and mode of activation in the ML/SL, the puffs I(B 77, 79), 3(C 85, 87), 4(D 91, 93), 4(E 17) and 5(B 57) could be seen as early puffs. 1-18-C in *C. tentans* (Clever 1962) and 4(E 17) and 5(B 57) in *A. lucidus* will be activated in the Malpighian tubules.
2. Puffs, which are already active in 4.LS, but reach their maximum size only with the increase in ecdysone titre and which regress until the end of PP, for example 1-17-B and 1-19-A. The same type of behaviour in the ML/SL of *A. lucidus* is shown by 2(B 73), 6(G 53) and 6(H 27-31).
3. Late puffs, which are formed towards the end of larval development in PP or after injection of ecdysone, after 48-72 h, for example I-l-A, 1-14-B, II-14-A and III-9-B. The same activation behaviour is shown by 2(G 19, 21), 3(B 15, 17), 3(F 73, 75), 4(B 95), 5(G 33, 35) and 6(C 73, 75) in ML/SL.
4. Puffs, which are already active in 4.LS and which show a strong increase in activity in mPP, for example 1-13-B, II-6-A. In ML/SL 3(A 47, 49) behaves in the same way.

The puffing patterns are more complex in the AL than in the ML/SL, for there at the same time, both synchronous and asynchronous changes occur. In an AL cell the asynchronous changes proceed considerably faster than the synchronous ones. Shortly after the regression of BR 3 and BR 4, a strong and rapid activation of the puffs 3(F 47), 3(G 25), 4(E 25, 27), 4(G 83, 85), 5(B 87, 89), 5(E 81, 83) and 6(G 43, 45) takes place. These asynchronous puffs, together with the synchronous puffs 1(B 77, 79), 4(E 17) and 5(B 57), could be called early puffs. All putative late puffs are

formed synchronously in all AL cells: 3(B 15, 17), 3(F 73, 75), 4(B 95), 5(B 17-21) and 6(C 73, 75).

In the previous classification only the largest puffs of *A. lucidus* (which reach size class 3) have been presented. Less active puffs can probably be classified similarly. During the period 4.LS - 1PP in AL ca. 40% and in ML/SL ca. 34% of the puffs change in activity. In *C. thummi*, where a very exact analysis of the puffing pattern has also been carried out, about 40% of the puffs show development- dependent changes in activity during the last two larval and the prepupal stages (Kiknadze 1981).

Asynchrony in changes in activity of distinct loci occurs in *A. lucidus* within one tissue, the AL (nucleolus, BR 3, BR 4 and some puffs), as well as between different tissues, salivary glands and Malpighian tubules (only the nucleolus). Of interest is that in both cases the nucleolus shows asynchrony. A gradient within the salivary gland (from distal to proximal) has also been observed in *Rhynchosciara holleanderi* during the activation of several DNA puffs (Stocker and Pavan 1977). Here it is remarkable that in the proximal part of the salivary gland, the active period of the DNA puffs is shorter than in the distal part. The same has been found for the asynchronous puffs in the AL base cells of *A. lucidus*. Another case of asynchrony in changes in puffing patterns has been observed by Trepte (1980) in *Sarcophaga barbata*. The puffs of the abdominal trichogen cells are activated 1 day later than those of the thoracic trichogen cells. Trepte has concluded that after hormonal stimulation the bristle cells control the timing of their puffing activities autonomously.

If cells differ in sensitivity to ecdysone and are successively induced as the ecdysone titre increases the formation of a gradient or a delay in puffing changes could be explained. That is not the case in the AL of *A. lucidus*, for here, after the injection of very high amounts of ecdysone in fourth instar larvae, no synchronous regression of all BRs was observed (Panitz et al. 1972) although the period of BR regression was shortened from some days (Fig. 1) to a few hours. The process was therefore strongly accelerated but the gradient seems to be predetermined.

The parallel occurrence of both asynchronous and synchronous changes in puffing within the AL, points to different mechanisms for each. Panitz (1964) reported that, in the AL of animals in j4.LS/PP, the regression of BRs, which had just started in the tip of the AL, continued to the base during cultivation of the gland in larval haemolymph, a milieu free of hormone. He assumed that, for initiation of regression of BRs, only very low amounts of hormone are necessary and that the further course of regression is controlled autonomously in the AL cells.

In experiments with *A. lucidus*, α-ecdysone was about twice as efficient as β-ecdysone (Panitz et al. 1972). In contrast in *Drosophila*, α-ecdysone showed only 2-3% of the efficiency of β-ecdysone in the induction of puffing changes. Of particular interest in this connection is the difference in sensitivity and reaction to α- and β-ecdysone of the early puffs 1-18-C and IV-2-B of *C. tentans* which Clever et al. (1973) demonstrated by injection and in vitro experiments. In vitro, 1-18-C could only be induced by α-ecdysone, not by β-ecdysone; IV-2-B reacts within a few minutes to β-ecdysone and to α-ecdysone with a considerable delay. Significantly, during normal development,

1-18-C is clearly activated before IV-2-B and α-ecdysone is the only ecdysteroid released by the prothoracic gland. Most probably I-18-C is induced by α-ecdysone in the larva.

From the previously cited results, it is possible that the initiation and the sequence of puffing in the AL of *A. lucidus* occurs as follows: the asynchronous regression of BRs is initiated by α-ecdysone. Each AL cell controls the timing of regression and the subsequent sequence of activation and inactivation of puffs autonomously. The tip to base gradient in AL is predetermined. The synchronous puffing changes are induced by the increasing concentration of β-ecdysone in the haemolymph. In ML/SL β-ecdysone should also induce the synchronous changes.

The fact that the asynchronous changes (in the tip cells of AL) precede the synchronous ones and that, as mentioned before, α-ecdysone is the primary released ecdysteroid, supports the assumption that α-ecdysone initiates the asynchronous changes. The rapidity of these changes supports the idea of an autonomous regulation of their timing in the individual AL cell.

The sequence of developmental changes in activity can be seen especially well in the apparent migration of the puff maximum along the BR 4 region (from distal to proximal) described by Mechelke (1961). The migration comes about by the sequential, asynchronous and synchronous, inactivation and activation of loci lying closely together but separated by inactive bands. Recently Semeshin et al. (1985) have given a detailed EM-based description of a similar movement of a puff maximum along a chromosomal region of *D. melanogaster*. In this case the migration was also caused by the sequential activation of some independent, closely neighbouring loci.

In *A. lucidus* a number of puffs first reach their maximal activity in 1PP. Most of these puffs are formed both in AL and in ML/SL. The assumption of a connection between these puffs and the catabolic processes of the subsequent histolysis of the salivary glands seems to be the best explanation.

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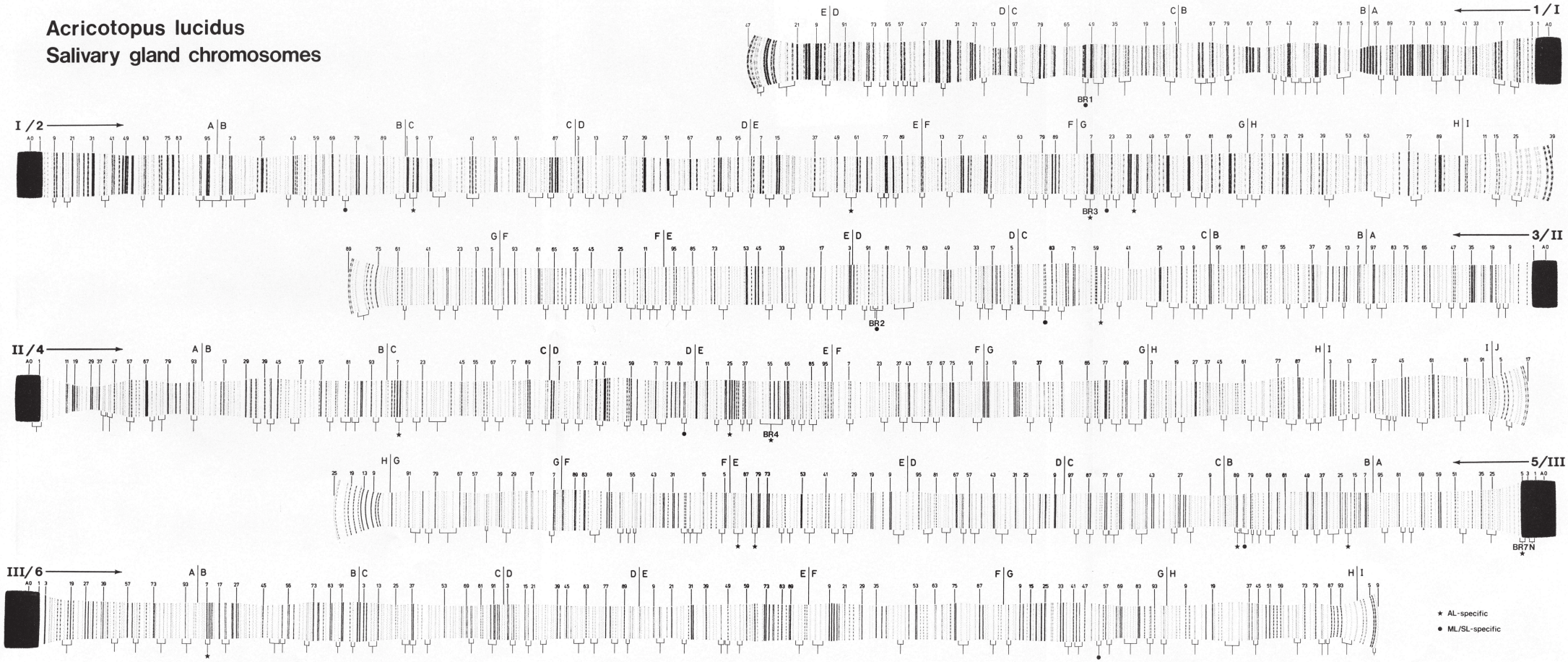
Acricotopus lucidus
Salivary gland chromosomes



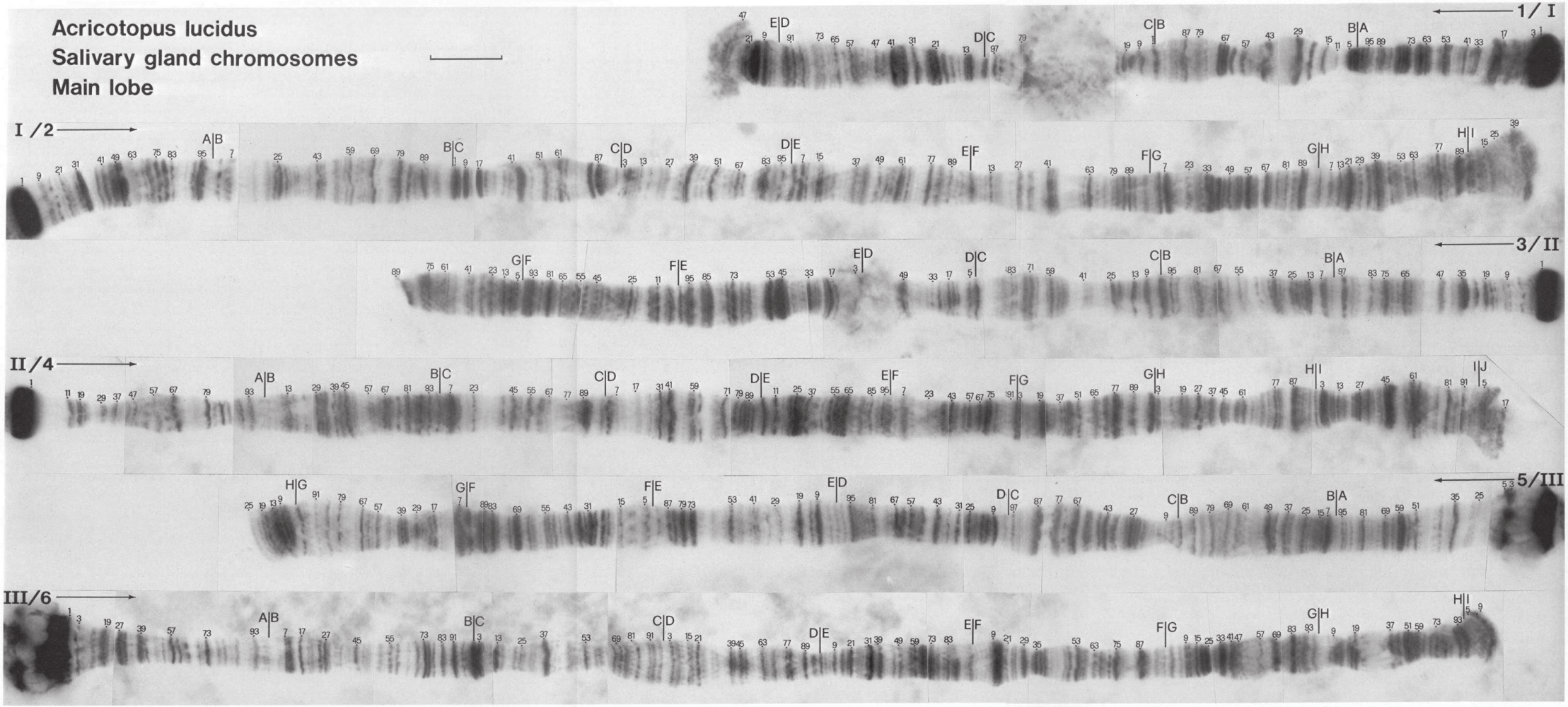


Plate II. Photographic map of the main lobe chromosomes with chromosome and band designations. The map was constructed from stretched chromosome sections from different animals in 4.LS/PP; only the condensed centromere/nucleolar organizer regions of III/5 and III/6 are from animals in jPP