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Patterns of puffing activity and chromosomal polymorphism in *Drosophila subobscura*

3. Puffing activity depression by inbreeding *

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Summary. The effect of inbreeding on the puffing patterns of polytene chromosomes of *Drosophila subobscura* was analysed. Puffing activity was studied in two strains of *D. subobscura:* one which had been subject to inbreeding for 288 generations, and in the hybrids from a cross between them. A strong overall decrease in puffing activity was found in the inbred line. In general, hybrids behaved in a similar way to the inbred line or showed activity intermediate between the two lines. The fertility and viability of the two homozygous lines and of the hybrids were also determined. These parameters of fitness are very low in the inbred line. Hybrids displayed intermediate behaviour.

Key words: Polytene chromosomes – Puffing patterns – Inbreeding – *Drosophila subobscura*

Introduction

It is a well established fact that puffs are the most active sites of gene activity on polytene chromosomes. Accumulated evidence shows that puffs follow a characteristic sequence throughout development. In general, individuals of one species show basically the same puffing patterns at specific developmental stages. Even individuals belonging to sibling species show similar puffing patterns (Ashburner 1972). These similarities in puffing activity between individuals of two closely-related but different species do not mean absolute gene identity. Some qualitative and quantitative differences in puffing have been observed, for instance, between *D. melanogaster* and *D. simulans* (Ashburner 1969 a, b). Some variability has also been found among individuals belonging to the same species. Thus, some differences have been observed between different strains of *D. melanogaster* (Ashburner 1969 c) or between cells belonging to the same gland (Berendes 1965; Belyaeva and Zhimulev 1974), or between chromosomes of *D. subobscura* carrying different gene arrangements (de Frutos and Latorre 1982). Intraspecific variability in puffing patterns may also occur between strains that differ in the level of inbreeding. A weakening in gene activity was found by Lychev (1965) in third instar unsynchronised larvae of *D. melanogaster* from an inbred line, this was especially observable on the X chromosome. Belyaeva and Zhimulev (1974) studied variation in the size of certain puffs of *D. melanogaster* X chromosomes in inbred lines. No decrease in puffing activity during inbreeding was found.

The present paper describes the puffing patterns found in two strains of *D. subobscura* which are homozygous for the standard gene arrangements. One of these (K228) is a known laboratory strain that has been subject to prolonged inbreeding. Cytological data on the hybrids obtained by crossing the two strains are also presented. The results support the hypothesis that the inbreeding gave rise to a generalized depression of gene activity. This paper is part of a more general study dealing with the gene expression of polytene chromosomes of *D. subobscura* related to the chromosome polymorphism of this species.

Material and methods

The strains of *D. subobscura* used in this study were H271 and K228. The H271 strain is homozygous for standard gene arrangements in the five acrocentric chromosomes of this species. This stock was obtained from a female captured in a natural population of South Finland. K228 is a highly inbred line. Some years ago it was inbred for 228 generations. Since then this strain has been maintained in the laboratory without

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inbreeding. K228 is homozygous for all standard gene arrangements except the J chromosome which carries a J_1 inversion. The hybrids were obtained by crossing H271 virgin females to K228 males.

Puffing patterns of the salivary gland chromosomes of H271 and K228 strains were established at five times around the beginning of prepupa formation. Samples of the third late instar larvae and of prepupae at 0, ½, 1½ and 2½ h were taken. The method described by Pascual et al. (in preparation) was used to obtain untimed third instar larvae and timed prepupae. The 0, ½, 1½ and 2½ times are correlated to the following morphological changes: 0 h – prepupa to the time of the eversion of the anterior spiracles; ½ h – prepupa to white pupa formation; 1½ h – to moderately pigmented pupa; 2½ h – to strongly pigmented pupa. Only 0 h prepupae were analysed in the hybrids.

Five nuclei were sampled from each of the 500 individuals analysed, and 50 individuals per developmental stage and strain. Twenty preparations of the hybrid polytene chromosomes were also examined. Dissection of salivary glands and cytological manipulations were carried out following the method described by de Frutos and Latorre (1982). The location of the puffs is based on the standard salivary gland chromosome map of Kunze-Mühl and Müller (1958). In order to determine the level of puffing activity, two criteria were taken into account: a) size of puffs, and b) frequency of appearance of each puff at every stage analysed. In relation to the size of puffs three levels were counted: (2) intermediate and large puffs, (1) small puffs and (0) no puffs. On the other hand, in many cases puffs were found within a gland which showed different levels of activity. With respect to this, two classes of puffs have been considered: puffs (+) and puffs (+/–). A puff may be considered as (+) when the five chromosomes show level (2) of puffing within a gland, or (+/–) when the level of puffing within a gland varies between (2) and (1) (in a few cases between (2) and (0)).

The maintenance and preparation of the strains to obtain synchronised individuals, as well as the cytological manipulations, were carried out in a thermoregulated room at 19 ± 1 °C.

Viability of H271 and K228 strains and of the H271/K228 hybrids

Measures of fertility. Females aged 10 to 12 days which had previously been given daily changes onto fresh food, laid eggs during a period of 6 h. The number of females used was 326, 220, and 226 in H271, K228 and in (H271/K228) hybrids, respectively. The total number of eggs (hatched or not) laid by each group of females was counted.

Estimates of egg-to-adult viabilities. Seven hundred larvae per strain were seeded without overcrowding (70 larvae per vial). Emerging adults were counted as well as pupae that failed to emerge.

Results

Data from puffing studies are summarized in the frequency histograms (Figs. 1–5). As described above, two classes of puffs were taken into account: (+) puffs that show activity (2) in the five chromosomes within a gland (black in the histograms) and (+/–) puffs, in which activity varies between (1) and (2) within a gland (white in the histograms). A total of 138 loci were observed to be active in the H271 strain, whereas in the inbred strain K228, only 96 loci appear as puffs. In

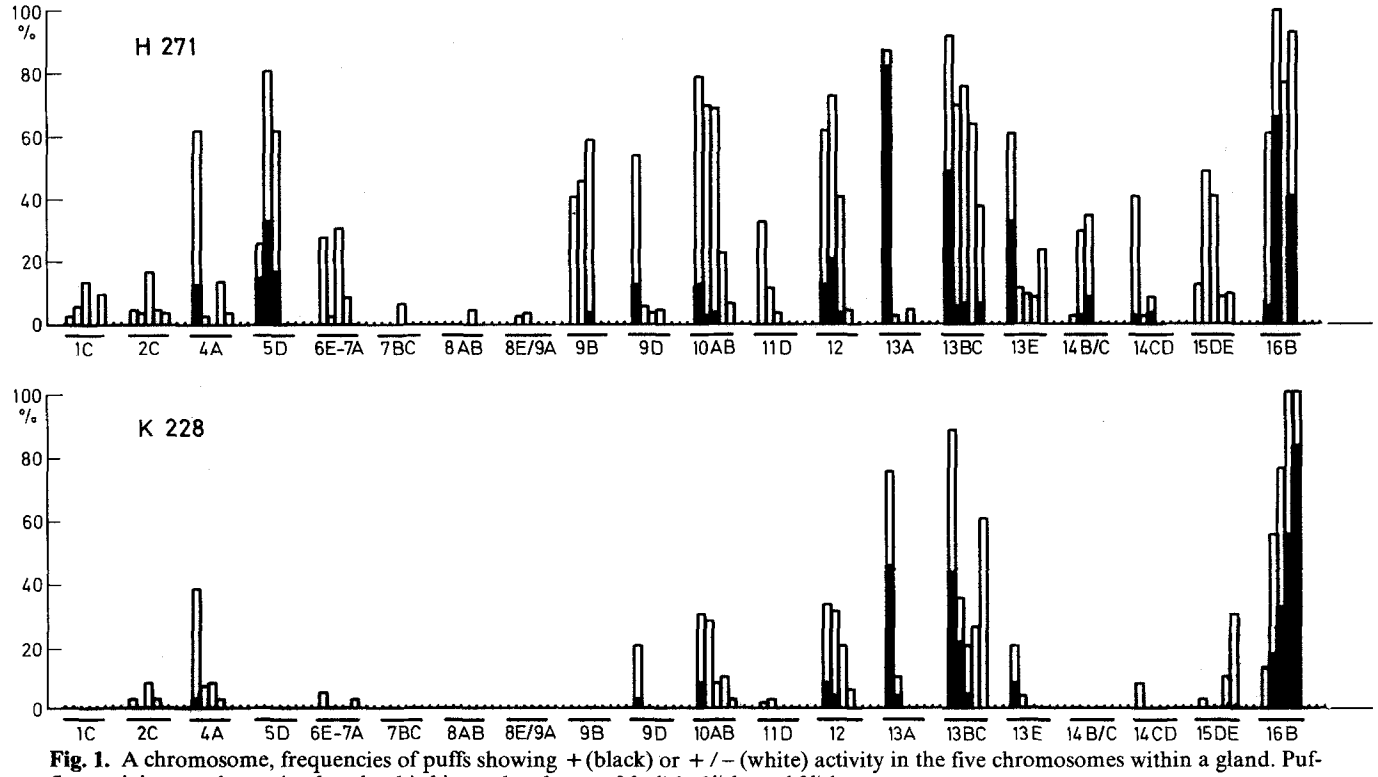

Fig. 1. A chromosome, frequencies of puffs showing + (black) or + / – (white) activity in the five chromosomes within a gland. Puffing activity was determined at the third instar late larvae, 0 h, ½ h, 1½ h, and 2½ h prepupae

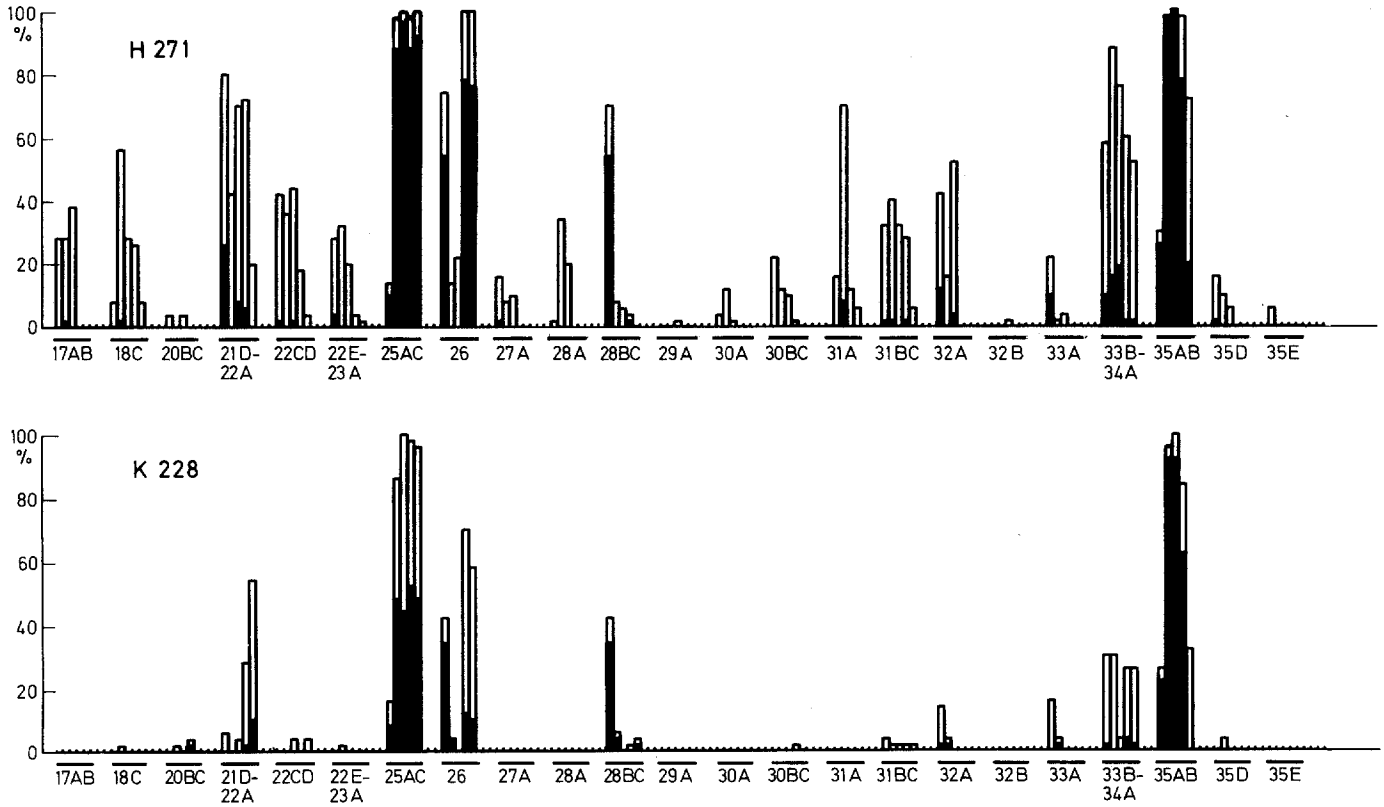

Fig. 2. J chromosome (legend as in A chromosome)

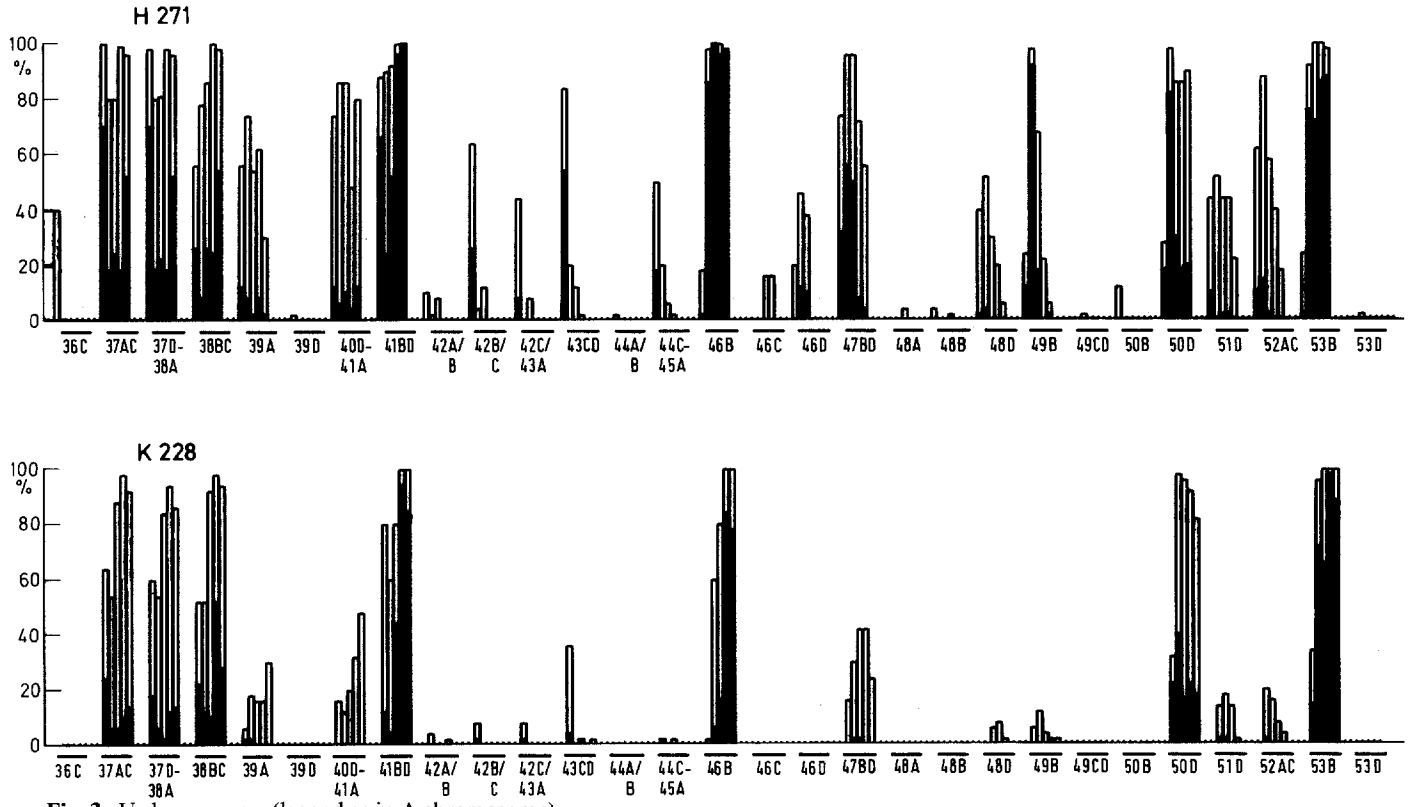

Fig. 3. U chromosome (legend as in A chromosome)

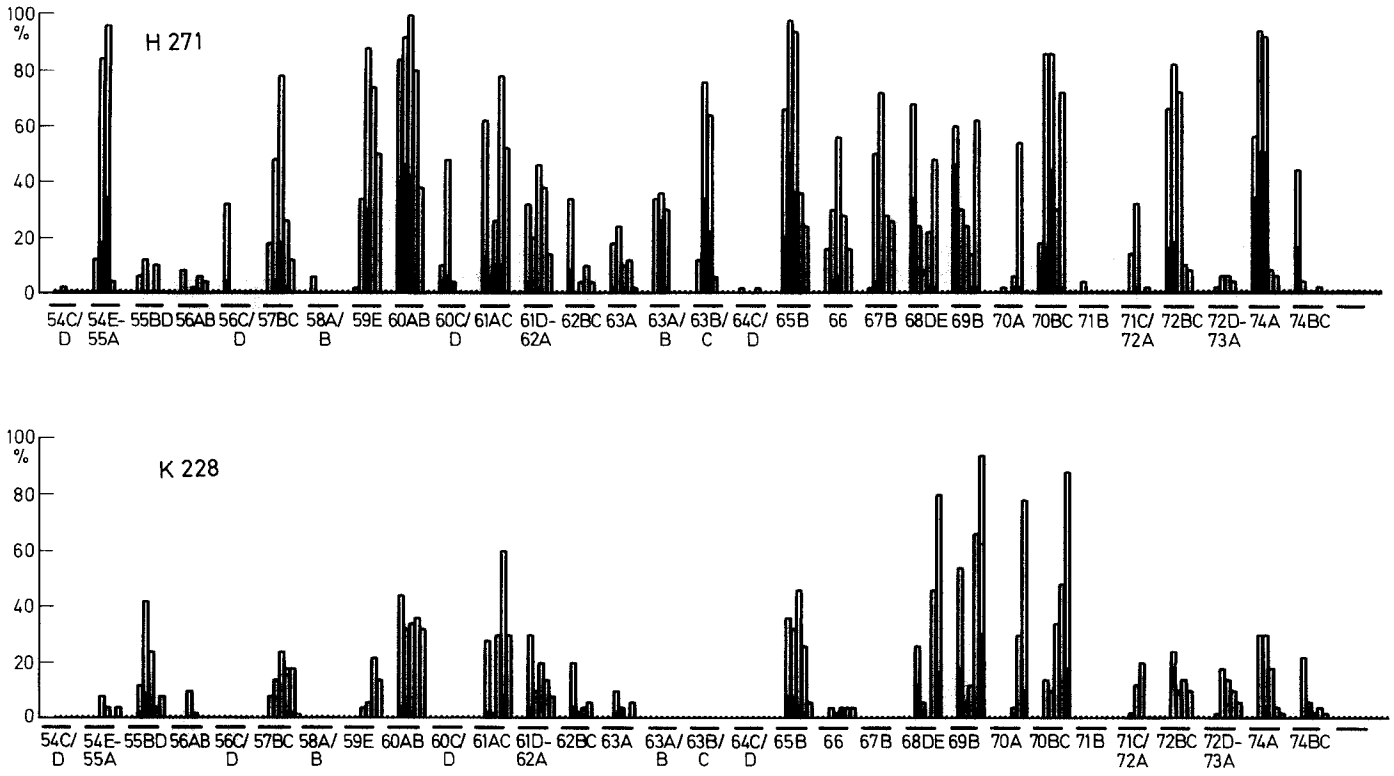

Fig. 4. E chromosome (legend as in A chromosome)

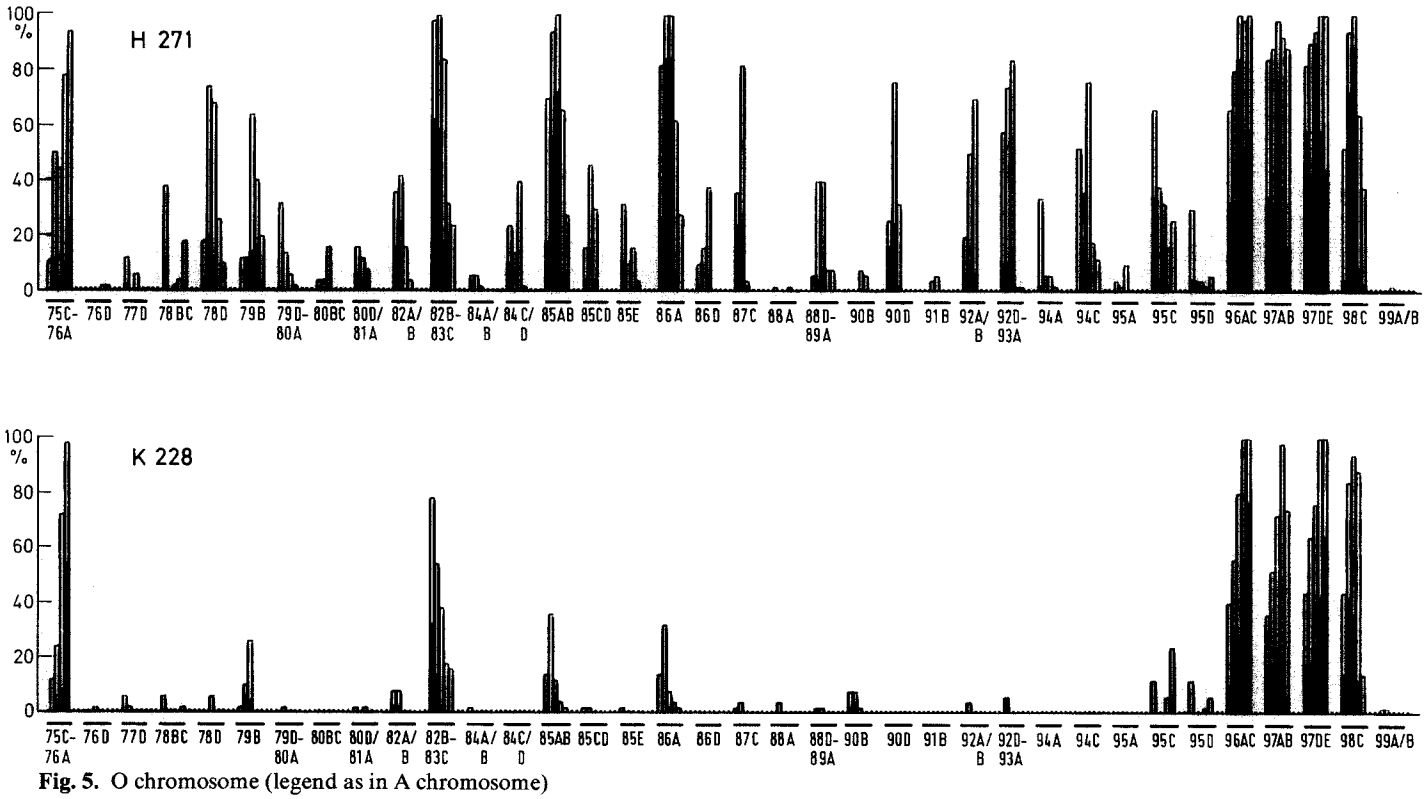

Fig. 5. O chromosome (legend as in A chromosome)

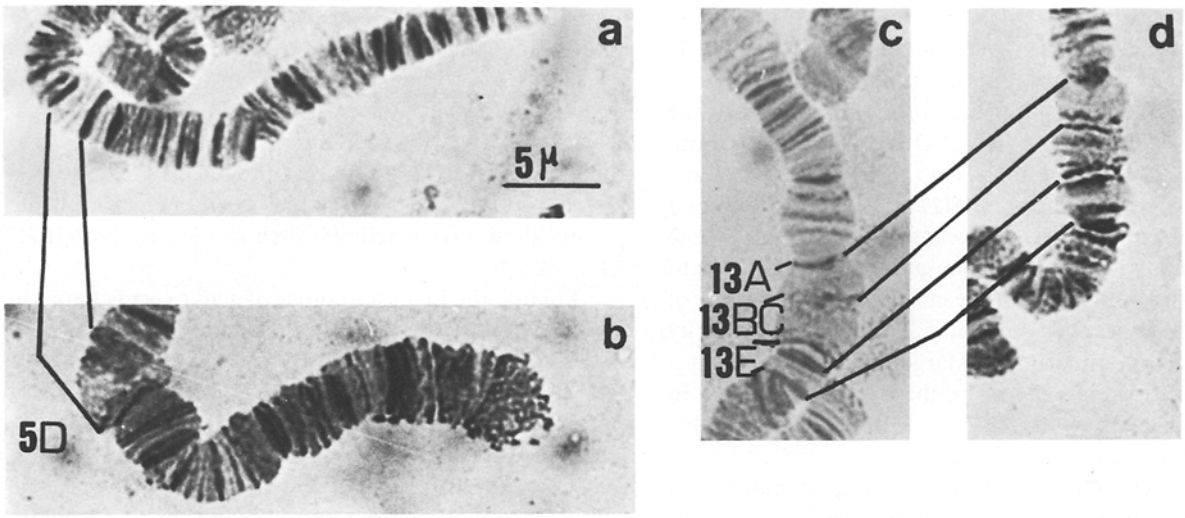

Fig. 6 a–d. A chromosome. **a–b** puff 5D. **a** K228 strain (0 h prepupa); **b** H271 strain (0 h prepupa); **c–d** puff 13E, **c** K228 strain (3rd instar larva); **d** H271 strain (3rd instar larva)

general, the puffs of K228 also show weaker activity than the H271 puffs, that is to say, the K228 puffs are smaller and show lower frequency than those of the H271 strain.

In the A chromosome, 20 puffs were found in the H271 strain. Of these, only 13 show activity in the K228 strain. Some of the puffs observed only in the H271 strain can be considered as occasional puffs. These puffs have a low frequency and are small. Others are more active, for instance 5D (Fig. 6a) and 9b. The puffs which are active in both strains tend to exhibit weaker activity in K228 than in H271, for example, 13E (Fig. 6b).

Twenty-three puffs are found in the J chromosome, of which 8 are exclusively active in the H271 strain. Except for 31A (Fig. 7) these 8 puffs can be considered as occasional puffs. As in the A chromosome, the active puffs in the two strains tend to show weaker activity in K228 than in H271. The number of puffs that can be included in the "characteristic pattern" is in general higher in H271 than in K228 strain. Those puffs that display a high frequency (more than 70%) at a given stage are considered here to constitute the "characteristic pattern". Thus, 21D–22A, 25AC, 26, 31A, 33B–34A, and 35AB form the "characteristic pattern" of H271 prepupae while in K228 prepupae only loci 25C and 35AB reach high frequencies. As can be seen in the histogram (Fig. 2), the shapes of the most active puffs generally show the same trend in both the K228 and H271 strains although gene activity is lower in the K228 strain. For instance, locus 26 decreases in activity between 0 h and ½ h. 28BC is active at the third instar and afterwards it decreases, 33B–34A is active at all stages analysed. In some cases, however, timing differences can be observed between the two strains, as in 21D–22A.

The U chromosome together with 26, 28C and 35AB of the J chromosome display the largest puffs of this species. The 10 loci that appear active exclusively in H271 can all be considered as occasional puffs. Strong differences have been observed in the puffing level of loci active in both strains. In the third instar larvae of K228 only 41BD can be considered to belong to the "characteristic pattern" and the number of chromosomes with level (2) activity is also low. In spite of this, a number of loci exhibit strong activity in the third instar larvae of H271 (37AC, 37D–38A, 40D–41A, 41BD, 43CD, 47BD). Noticeable activity can also be observed at this stage in sections 42, 43 and 44 of H271,

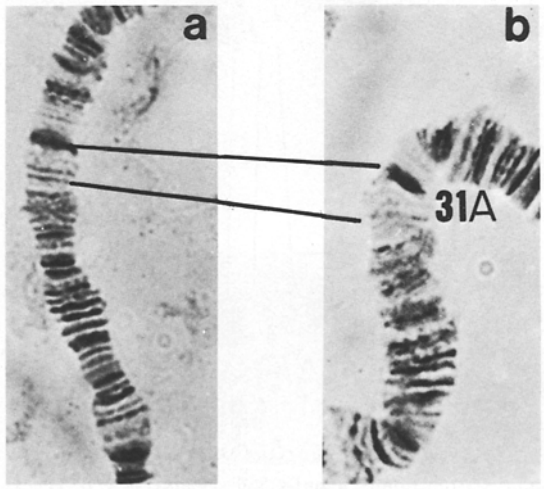

Fig. 7 a, b. J chromosome, puff 31A: **a** K228 strain (0 h prepupa); **b** H271 strain (0 h prepupa)

while practically no activity is found in K228. It could be considered that two zones of activity exist on the U chromosome at the beginning of prepupation. A set of loci located at the end of the chromosome near to the centromere (from 37AC to 41BD) show activity in both strains at every stage analysed, although in K228 the activity is lower than in H271. It is possible that "housekeeping" genes are located in these regions, the products of which are indispensable for cellular metabolism. At the other end of the chromosome a number of puffs located between 46B and 53D show strong activity in the H271 strain. They are large puffs which confer a characteristic appearance to the U chromosome. In spite of this, only 46B, 50B and 53B display noticeable puffing activity in the K228 strain.

The E chromosome exhibits a higher number of active loci than the chromosomes described above. However, the puffs are small and generally appear with low frequency. In the K228 strain the puffing activity of the E chromosome is really low. For example, no puffs reach values over 70% at the third instar, and in prepupae only the four adjacent puffs, 68DE, 69B, 70A and 70BC, show strong activity. Apart from these puffs the level of puffing activity is very low. Many E chromosomes at 0 h prepupae show no puffs, that is to say they are chromosomes with a complete absence of puffing activity. In the H271 strain puffing activity is clearly greater than in K228. For example, 9 puffs reach values over 70% at the prepupal stage, while in K228 only four do so. With respect to the puffs that only appear in H271, some of these can be considered as occasional, such 56C/D and 58A/B (Fig. 8a), while others show strong activity, such as 63A/B and 63B/C (Fig. 8c).

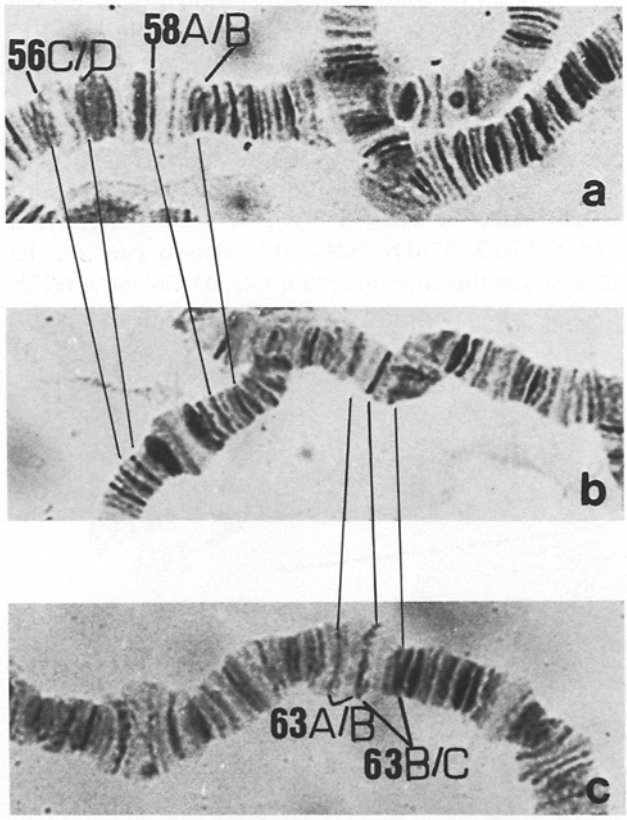

Fig. 8a–c. E chromosome. **a** puffs 56C/D and 58A/B, H271 strain (3rd instar larva); **b** K228 strain; **c** puffs 63A/B and 63B/C, H271 strain (0 h prepupa)

Finally, the highest number of active loci is found in the 0 chromosome, which is also the largest chromosome. The strong differences between the two strains can be seen in Fig. 5. In the K228 strain at the third instar only 82B–83C shows a high level of activity. 75C–76A, located on the proximal end of the chromosome, and 96AC, 97AB, 97DE and 98C, located on the distal end of the chromosome, make up the "characteristic pattern" at the prepupal stage. Practically no gene activity has been found outside these loci. In contrast, strong activity was observed in the H271 strain throughout the chromosome. The activity on loci 85AB and 86A is worth mentioning.

The coefficients of correlations (r) between the puffing percentages of the two strains were estimated for each stage and chromosome. In A, J, U and 0 chromosome r values there are no significant differences at the five stages analysed, while in the E chromosome no correlation at 0 and ½ h prepupae was found.

The values of the A index are represented in Fig. 9. The A index gives the average gene activity per stage and chromosome (Pascual et al., in preparation).

$$A = \frac{1}{N} \sum_{i=1}^{i=N} P_i$$

where:

N is the number of puffs described in a given chromosome

P is the frequency of puffing in a given locus.

The remarkable differences in activity between the two strains can be visualized from the index values. Puff activity is markedly lower in the K228 chromosomes than in the H271 ones. U is the most active chromosome in both strains. A marked peak of activity in 0 or ½ h prepupae appears in H271, except in the A chromosome. In this chromosome a peak of maximum activity is found in the third instar larvae. This behaviour agrees with that found in other strains of *D. subobscura* (Pascual et al., in preparation). A, J and U chromosomes show similar behaviour in the two strains, although in K228 the activity is lower than in H271 and a displacement to the right of the maximum peak of activity is found in K228. In the E and 0 chromosomes of the K228 strain the level of gene activity varies a little, and is drastically weaker than in

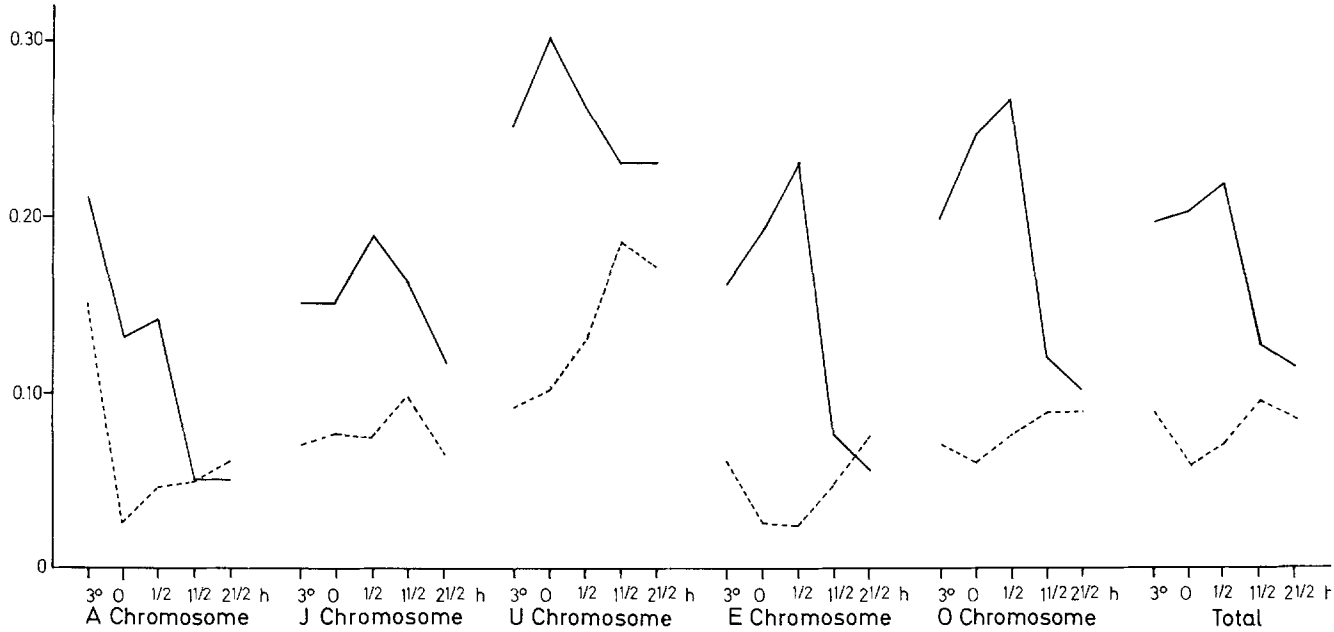

Fig. 9. A index values. *Solid line* H271 strain, *dotted line* K228 strain.

the same chromosomes of H271. In this last strain a strong drop in gene activity is found in ½ h prepupae. At this time the level of puffing is similar and very low in both strains. In considering the A index relative to all chromosomes as a whole, the following results can be extracted: in K228 puff activity is low and without large changes, in H271 puff activity is high and shows a maximum of activity in ½ h prepupae.

Puff frequencies in 0 h hybrid prepupae from the cross of H271 with K228 are given in Table 1, together with those of H271 and K228 for the same stage. In general, either puffing patterns in hybrids are similar to those of K228, or they show an intermediate behaviour between H271 and K228. In some cases, however, puffs exhibit the same activity in the hybrids and in the two homozygous strains: for example 16B of A chromosome, 25AC and 35AB of J chromosome, 37AC, 37D–38A, 38BC, 46B, 50D and 53B of U chromosome.

The results on the viability of homozygous strains and hybrids are given in Table 2. Female fertility (number of eggs laid per female and per hour) is very low in K228, and a certain dominance of K228 appears in the hybrids. Similarly, total viability (% of adults emerged) is very low in K228. The number of adults that emerge per female and per hour was obtained by multiplying total viability per fertility. Only 0.0877 adults emerged per hour and K228 female compared with 0.7032 adults per hour and H271 female. This very low viability value of the K228 strain is fundamentally due to a high mortality in the eggs (37.89% in K228 and 8.74% in H271). A certain dominance of the H271 strain can be found in the viability of the hybrids. However, when considering the number of adults per hour and hybrid female, viability is similar to that found in K228.

Discussion

The primary effect of inbreeding is an overall increase in homozygotes. A second effect is that inbreeding often leads to a depression in the expression of several traits, especially of traits pertaining to fitness. It is evident that this phenomenon leads to the phenotypic expression of lethal or deleterious alleles because it reveals the variation hidden by the dominance of "normal" alleles. The data presented here indicate that inbreeding leads to a decrease in puffing activity in a highly inbred line of *D. subobscura.*

Lychev (1965) found similar results in *D. melanogaster.* This author analysed puffing activity in untimed third instar larvae of a line which had been inbred from the 36th to the 41st generations, and compared the results with puffing patterns of *D. melanogaster* lines without inbreeding. A general tendency towards decreased puffing activity in the inbred line was observed. However, it cannot be precisely determined to what extent the decrease in gene activity occurs since the data are not quite comparable because individuals are not synchronised. On the other hand, Belyaeva and Zhimulev (1974) studied variation in size of puffs of the *D. melanogaster* X chromosome at 0 h prepupae from lines both with and without inbreeding. No noticeable effect of inbreeding on the size of puffs was found. The differences in puffing between the two *D. subobscura* lines described in this paper are large. However, these differences may be due to the strains rather than to inbreeding. Puffing activity in other *D. subobscura* stocks which are homozygous for different gene arrangements and were obtained, as was H271, from individuals captured in natural populations, is similar to that of H271 and manifestly higher than that found in K228 (de Frutos and Latorre 1982; Latorre et al., in preparation).

Table 1. Frequencies (%) of puffs showing (+/−) activity of H271, H271/K228 and K228 (0 h prepupa)

	H271	H271/K228	K228
A Chromosome			
1C	6.1	0	0
2C	3.6	10	2.5
4A	3	0	6.9
5D	81.8	0	0
6E–7A	3	0	5
8E/9A	3	0	0
9B	45.5	10	0
9D	6.1	0	0
10AB	69.7	20	27.6
11D	12.1	0	0
12	72.7	20	31
13A	3	0	10.3
13BC	69.7	10	34.5
13E	12.1	0	3.5
14B/C	30.3	0	0
14CD	3	0	0
15DE	48.5	10	0
16B	60.6	80	55.2
E Chromosome			
54E–55A	84	85	8
55BD	12	15	42
57BC	48	50	14
59E	34	75	4
60AB	92	75	32
60C/D	48	0	0
61AC	6	20	2
61D–62A	20	15	10
62BC	0	0	2
62D–63A	24	0	4
63A/B	36	5	0
63B/C	76	5	0
65B	98	75	32
66	30	10	2
67AB	50	40	18
68DE	24	10	6
69B	30	45	6
70A	2	15	0
70BC	86	85	10
71C/72A	14	50	12
72BC	82	50	10
72D/73A	2	50	18
74A	94	80	10
74BC	4	0	6
J Chromosome			
17AB	28	0	2
18C	56	5	2
20BC	0	0	2
21D–22A	42	0	0
22CD	36	0	0
22E–23A	32	0	2
25AC	98	90	86
26	14	0	4
27A	8	0	0
28A	43	0	0
28BC	8	0	6
30A	12	5	0
30BC	12	5	0
31A	16	0	0
31BC	40	0	2
32A	16	0	4
33A	2	0	4
33B–34A	88	45	30
35AB	98	100	96
U Chromosome			
37AC	80	85	54
37D–38A	80	85	54
38BC	78	85	52
39A	74	70	18
39D	2	0	0
40D–41A	86	40	12
41BD	90	85	60
42A/B	2	0	0
42B/C	4	0	0
43CD	20	0	0
44A/B	2	0	0
44C–45A	20	0	0
46B	96	100	60
46D	46	10	0
47BD	96	70	30
48D	52	10	6
49B	98	50	12
50D	98	90	98
51D	52	45	18
52AC	88	35	16
53B	92	100	96
O Chromosome			
75C–76A	50	35	12
77D	2	0	0
78D	74	40	0
79B	12	45	10
79D–80A	14	0	0
80BC	4	0	0
80D/81A	12	0	0
82A/B	42	0	2
82B–83C	100	75	54
84A/B	6	0	0
84C/D	14	0	0
85AB	94	75	36
85CD	46	0	2
86A	100	85	32
86D	16	0	0
87C	82	0	4
88D–89A	40	0	0
90B	0	5	0
90D	76	15	0
91B	4	0	0
92A/B	50	15	0
92D–93A	74	20	6
94A	6	0	0
94C	36	0	0
95A	2	5	0
95C	38	10	0
95D	4	0	0
96AC	80	90	56
97AB	88	80	52
97DE	90	90	64
98C	94	100	84

Table 2. Fertility and viability of H271 and K228 strains and the H271/K228 hybrids

	Fertility[a]	Mortality (%)			Viability (%)	Adults per ♀ and per hour
		Eggs	Larvae	Pupae		
H271	1.0414	8.74	19.69	4.04	67.53	0.7032
H271/K228	0.3820	13.71	24.41	2.71	59.17	0.2260
K228	0.2439	37.89	24.84	1.33	35.94	0.0877

[a] Fertility = number of laid eggs/number ♀♀/number of hours

Differences in puffing between the two strains can be considered from several points of view.

1 Differences in the number of active loci

One hundred and thirty-eight puffs were found in H271 and only 96 in K228. The larger number of the puffs that only appear in H271 are occasionals, but in some cases they show strong activity (for example, 5D of A chromosome, 31A of J chromosome, 63A/B and 63B/C of E chromosome). This important decrease in the number of active puffs can be correlated with the low viability values of the K228 strain. The number of adult individuals per hour and female is 0.0877 while in H271 it is 0.7032. This low viability value is mainly due to the high mortality at the egg stage (37.89% in the K228 strain, and 8.74% in H271). On the other hand, the depression of viability due to inbreeding has been found in several species of *Drosophila* (Dobzhansky et al. 1963; Mettler et al. 1966). The high mortality at the egg stage could be a consequence of lethal genes that would come into action at this developmental stage. Lethality could to some extent be due to a negative control of gene expression, that is to say, it could be due to the non-expression of specific loci in the course of development.

2 Puffs active in both strains but which exhibit a lower activity in K228 than in the H271 strain

The greater number of the puffs described in this paper belong to this group. This decrease in gene activity is similar to that found by Lychev (1968) in *D. melanogaster*. If the degree of puffing is proportional to the degree of transcription, an overall process of slowing down of transcription would be found as a consequence of inbreeding. It is obvious that if inbreeding leads to a depression in the level of the functionality of individuals, in a similar way it would lead to an overall depression of gene activity. But this leads to the question of how an increase in lethal gene expression could imply a general decrease in transcription efficiency. A generalized restriction of RNA synthesis has been found in other biological situations, for instance in the stringent control in bacteria. Bacterial cells respond stringently, for example, in hard times incurred during amino acid deprivation. A reduction in the global rate of RNA synthesis could then occur – among other phenomena (Gallant 1979).

3 Puffs active in both strains which show a similar degree of puffing

A certain number of puffs that show a high frequency in each stage have been found. These puffs, whose frequency is higher than 70% at a given stage, were considered to comprise the "characteristic pattern". The number of puffs which constitute the "characteristic pattern" in each of the chromosomes and stages analysed of K228 strain is noticeably lower than that found in the H271 strain. In a more restrictive sense it could be considered that those puffs which reach frequencies higher than 70% in both strains make up the "characteristic pattern" for a given stage. In this sense the "characteristic pattern" of prepupae is constituted by: 16B (A chromosome), 25AC and 35AB (J chromosome, 37AC, 37D–38A, 38C, 41BD, 46B, 50D, and 53B (U chromosome), 68DE, 69B, 70A, and 70BC (E chromosome) and, 75C–76A, 96C, 97AB, 97DE and 98C (0 chromosome) (Fig. 10). This group shows a similar activity (size and frequency) in both strains. These results agree with those found for the X chromosome of *D. melanogaster* obtained by Belyaeva and Zhimulev (1974). In fact, these puffs show a quite invariable pattern in all *D. subobscura* strains analysed (de Frutos and Latorre 1982; Latorre et al. 1983; Pascual et al., in preparation). It can be concluded that this little group of puffs constitutes the pattern of gene activity indispensable for cellular life at the beginning of prepupal formation.

The values of the A index give the average puffing activity (frequency) per chromosome and developmental stage. For the five chromosomes the mean gene activity is lower in K228 than in H271. The values of the A index relative to the H271 strain are similar to those found for other *D. subobscura* strains (Pascual et al., in preparation). To summarize, in the inbred line K228, puffing activity, as well the activity of single puffs as the mean activity per chromosome, is markedly lower than in other, non-inbred lines.

The results from the hybrids are not accurate, hybrids were only studied at 0 h prepupae. A small group of puffs show similar behaviour in both the hybrids and in the two homozygous strains. These are the same puffs that are considered, in a strict sense, to be the "characteristic pattern". The results from the hybrids show the invariable behaviour that puffs of this group generally show, and reaffirm their importance as the basic gene pattern for cellular life. The large number of the hybrid puffs behaved in a different way. If depression of gene activity and viability is a consequence of an increase in recessive deleterious traits in the homozygous condition, the hybrid must be expected to behave as the homozygous without inbreeding. Hybrids would at least be expected to behave as a non-inbred line. However, the results obtained do not agree with this hypothesis. Puffs found in the hybrids generally show either a similar activity (size and frequency) to the homozygous strain K228 or an activity intermediate between H271 and K228. Analogous results have been found for hybrid viability. Possibly the overall decrease in gene activity due to inbreeding is a consequence of a phenomenon of regulation that

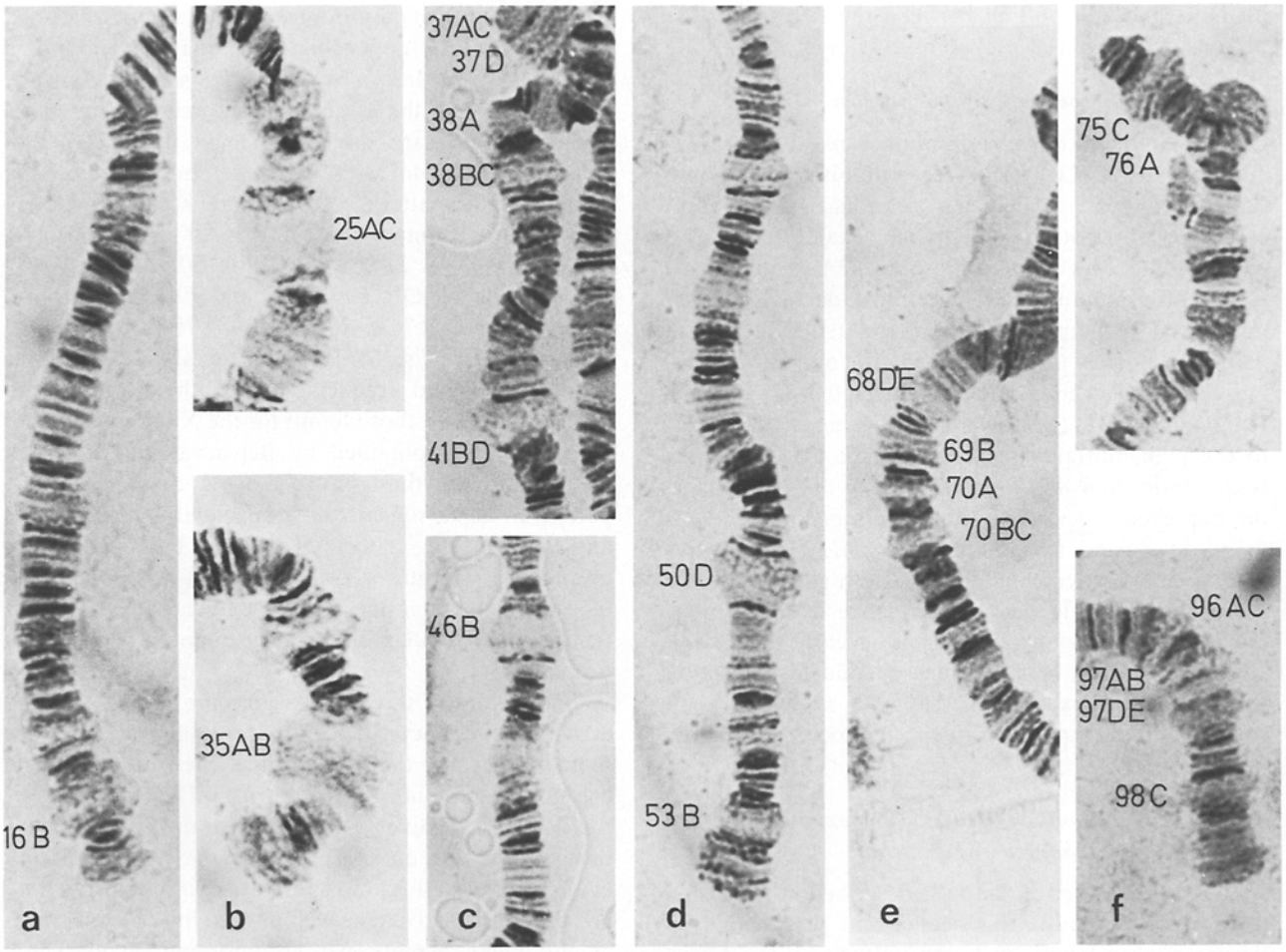

Fig. 10. **a** Puff 16B of A chromosome, K228 strain (0 h prepupa); **b** puffs 25AC and 35AB of J chromosome, H271 strain (0 h prepupa); **c** puffs 37AC, 37D-38A, 38BC, 41BD and 46B of U chromosome, K228 strain (0 h prepupa); **d** puffs 50D and 53B of U chromosome, K228 strain (0 h prepupa); **e** puffs 68DE, 69B, 70A and 70BC of E chromosome, K228 strain (0 h prepupa); **f** puffs 75C–76A, 96AC, 97AB, 97DE and 98C of O chromosome, K228 strain (0 h prepupa)

would implicate the whole genome rather than an accumulation of deleterious genes in homozygosis.

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