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# Characterization of a novel *Minute*-locus in *Drosophila melanogaster*: a putative ribosomal protein gene

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**We describe a novel *Minute* locus, *M*(1)7*C*, on the X-chromosome of *Drosophila melanogaster*. Heterozygous deficient females have most, if not all, of the *Minute* features (short and fine bristles, rough and somewhat larger eyes, thin-textured wings, missing aristae, affected antennae, delayed development, reduced fertility, and decreased viability). Both *Minute* and non-*Minute* adult progeny from *Minute* mothers suffer from *Minute* maternal effects such as abdominal segmentation defects, fused tergites, and missing or defective legs and halteres. Using a plasmid clone from region 7C5-9, which harbours the *D. melanogaster* ribosomal protein gene *RPS*14, we have found that the accumulation of a single transcript of ~650 b is extremely reduced in *Minute* larvae in comparison with wild-type. We have localized the *RPS*14 gene to ~28 kbp distal from the *singed* locus. The results suggest that *M*(1)7*C* and *RPS*14 may be the same gene.**

## INTRODUCTION

The *Minute* mutations of *Drosophila melanogaster* are a class of 40–50 loci that cause thin, short bristles, delay development time, reduce female fertility, decrease viability, produce rough eyes, small body size, thin wings, and etched tergites (Lindsley and Grell, 1968). In homozygous or hemizygous condition, *Minute* mutants are lethal and die at about the time of egg hatching. A heterozygous deficiency for a *Minute* locus produces a *Minute* phenotype.

It has been suggested that the *Minute* loci are the loci for tRNAs (Ritossa *et al.* 1966) or for ribosomal proteins (for review see Kay and Jacobs-Lorena, 1987). In the course of cloning the *dec-1* locus (cytological position 7C3-4; A. Lambertsson, T. Johansson, and S. Andersson, unpublished results) by a chromosome walk from the *singed* locus (1-21·0; 7D1-2) we observed that several heterozygous deficiencies uncovering the region 7C5-9 produced *Minute* phenotypes. This interval of the X-chromosome has been reported to contain a haplo-lethal locus (Lefevre Jr. and Johnson, 1973), and a ribosomal protein gene, *RPS*14 (Brown *et al.*, 1988). It has been suggested that *Minutes* code for ribosomal proteins (Burns *et al.*, 1984; Vaslet *et al.*, 1980) but only one, *M*(3)99*D*, has been shown to do so (Kongsuwan *et al.*, 1985). We therefore decided to examine this region for the presence of a *Minute* locus. The results presented here show that a novel *Minute* locus, *M*(1)7*C*, is located in the 7C5-9 region, and that the accumulation of the *RPS*14 mRNA is extremely reduced in *Minute* larvae in comparison with wild-type.

## MATERIALS AND METHODS

### *Drosophila stocks and fly handling*

Flies were raised on standard potatomash, yeast and agar substrate at various temperatures—17°, 25°, 29°C as well as room temperature (20–23°C).

The haplolethal (*hl*)-deficiencies listed below were a kind gift from Dr M. Gans. They were originally recovered in an X-ray screen performed by Dr F. Forquignon. Details about the $Dp(1;2)sn^{+72d}$ strain can be found in Lindsley and Zimm (1987). The wild-type stock used here was Shahrinau, obtained from the European Drosophila Stock Center, Umeå, Sweden.

$C(1)DX, yf/w\,cv\,Df(1)hl^{a}\,B;$
$Dp(1;2)sn^{+72d}/bw^{D}$
$C(1)Dx, yf/Df(1)hl^{b};\ Dp(1;2)sn^{+72d}/bw^{D}$
$C(1)DX, yf/Df(1)hl^{c};\ Dp(1;2)sn^{+72d}/bw^{D}$
$C(1)DX, yf/cm\,Df(1)hl^{d}f;$
$Dp(1;2)sn^{+72d}/bw^{D}$

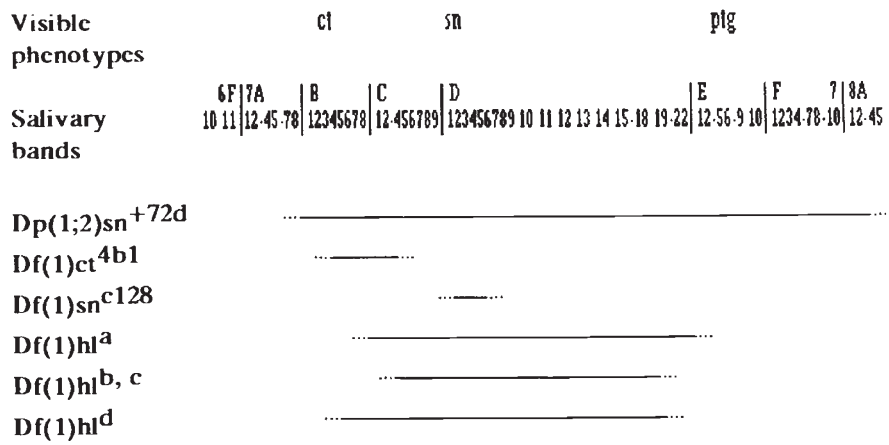


**Figure 1** Schematic representation of cytological region 6F10-11 to 8A1-5 on the X-chromosome also indicating the positions of *ct* = *cut*, *sn* = *singed* and *ptg* = *pentagon*, and the approximate extensions of the duplication and deficiencies used in this study.

The approximate lengths of these deficiencies and other chromosal rearrangement used in this study are given in fig. 1.

### *Crosses*

Shahrinau females, 2–5 days old (five in each tube), were mated with males carrying an *hl*-deficiency and the duplication covering the 7C5-9 region (see above). After 2 days eggs were collected for 2–4 h in tubes with fresh substrate. After discarding the parents the tubes were kept at the temperatures indicated. Of the G1 females those carrying the duplication hatched normally whereas the deficiency heterozygotes, phenotypically $bw^D$, hatched several days later. The latter females were collected and further examined for typical *Minute* characters.

### *Northern Blot Analysis*

Total RNA from wild-type and *Minute* larvae, and wild-type ovaries was prepared and fractionated on 1 per cent agarose-formaldehyde gels as described previously (Hansson and Lambertsson, 1983). Due to their delayed development, *M*(1)7*C* larvae are easily recognized in a synchronized larval population. All blotting onto GeneScreen Plus filters (Dupont, NEN Research Products) was carried out using the VacuGene Vacuum Blotting System (Pharmacia LKB Biotechnology AB).

The filter was hybridized at 60°C for 12–18 hr in 1 per cent SDS (sodium dodecyl sulphate), 1 M sodium chloride, 10 per cent dextran sulphate, and 200 μg/ml of sonicated and denatured salmon sperm DNA. Final concentration of the probe was $4 \times 10^5$ cpm/ml.

After removal of the filter from hybridization solution it was washed in 2 × 100 ml of 2 × SSC (0·3 M sodium chloride and 0·03 M sodium citrate) at room temperature for 5–10 min with constant agitation. Next, the filter was washed in 2 × 200 ml of 1 per cent SDS and 2 × SSC at 60°C for 30 min with constant agitation, and finally in 2 × 100 ml of 0·1 × SSC at room temperature for 30 min with constant agitation.

### *Probes*

The duplicated *D. melanogaster* ribosomal protein gene *RPS*14, cloned in Bluescript M13+ (pGS14-19), was used as probe. It was generously provided by Drs D. Rhoads and D. Roufa. pRH0·75 is a pBR322 plasmid clone containing most of the *Sgs*4 gene (salivary gland secretion; Hansson and Lambertsson 1983). The α-tubulin probe, which is cloned in pGEM-2, was kindly supplied by Dr M. Bownes. The probes were labeled with $^{32}$P-dCTP by primer extension following the supplier's instructions (Amersham or Promega Biotech).

## RESULTS AND DISCUSSIONS

### Minute *phenotypes produced by 7C5-9 heterozygous deficiencies*

#### *1. Decreased viability*

As shown in fig. 1 the *hl*-deficiencies used in this study have different lengths. $Df(1)hl^{b,c}$ are

**Table 1** Viability of offspring from crosses between Shahrinau wild-type females and $Df(1)hl^{b}$ or $Df(1)hl^{c}$ males; for complete genotypes see *Materials and Methods*. The data from the crosses are pooled

| | | Adults eclosed | | | |
|---|---|---|---|---|---|
| Eggs laid | Larvae hatched | $+/Df(1)hl^{b,c}$; $+/Dp(1;2)$ | $+/Df(1)hl^{b,c}$; $+/bw^{D*}$ | $+/Y$; $+/Dp(1;2)$ | $+/Y$; $+/bw^{D}$ |
| 747 | 692 | 173 | 59 | 134 | 173 |

* These females are *Minute*.

approximately of the same size and smaller than $Df(1)hl^{a,d}$ (M. Gans, personal communication). However, the exact positions of the breakpoints of these deficiencies are not known at the present time.

Having observed that crosses between *hl*-deficient males carrying $Dp(1;2)sn^{+72d}$ and wild-type females produced very late-hatching heterozygous deficient $bw^{D}$ females we decided to analyze further the nature of these females from each of the four *hl*-deficient males.

Males from each of the four *hl*-deficiencies carrying $Dp(1;2)sn^{+72d}$ were mated to wild-type females as described in *Materials and Methods*. Crosses involving $Df(1)hl^{a,d}$ males produced very few $bw^{D}$ females, and this is very likely due to the larger extension of these deficiencies in comparison with $Df(1)hl^{b,c}$ (see below).

In crosses at 25°C between Shahrinau wild-type females and males carrying $Df(1)hl^{b}$ or $Df(1)hl^{c}$ 92 per cent of the eggs laid hatched as larvae, and 72 per cent eclosed as adults (table 1). As shown in table 1 the number of *Minute* ($bw^{D}$) females is only 1/3 of that of the wild-type females carrying $Dp(1;2)sn^{+72d}$ or 25 per cent of the females ($n = 232$), and this explains the reduced number of flies hatched. In addition, the number of males carrying the duplication is slightly reduced in comparison to the $+;bw^{D}$ males (table 1). This is very likely due to reduced viability of the former males, which have been shown to be almost completely sterile (Gans *et al.*, 1980). We also noted that females being $bw^{D}$, and thus heterozygous for the deficiency, hatched considerably later when compared to wild-type or deficiency-heterozygotes carrying $Dp(1;2)sn^{+72d}$ (see below under *Development*).

To study further the viability of *Minutes* the crosses described above were repeated at 17°, 25°, and 29°C. The results from these crosses are summerized in fig. 2. At 25°C (fig. 2B) the number of *Minute* females is 1/3 of the wild-type females carrying the duplication or 25 per cent of the flies hatched ($n = 64$), and this is in accordance with the results described above (table 1). Surprisingly, however, at 17°C (fig. 2(A)) the *Minute* flies constitute 41 per cent ($n = 87$) of the females, and at 29°C (fig. 2(C)) 42 per cent ($n = 100$). Speculating that the *Minute* locus in 7C5-9 codes for a ribosomal protein or some other component of the translational machinery, this result is very interesting in that *Minute* flies would be expected to have reduced protein synthesis rates at higher temperatures. Consequently, the viability would be higher at lower temperatures and not at 29°C as observed here. We are presently planning experiments to study further this rather unexpected result.

When examining the progeny of $Df(1)hl^{a,d}$ males, crossed to Shahrinau females, we found that they produce non-*Minute* adults in the expected ratio. However, no adult *Minute* progeny emerged simply because the pupae died at different stages during pupal development. Since these deficiencies extend both proximally and distally (7B3-4; 7E1-2) relative to $Df(1)hl^{b,c}$, it is possible that they uncover a pupal lethal(s) in these regions of the Shahrinau X-chromosome. It should be mentioned, however, that $Df(1)ct^{4bl}/Df(1)sn^{c128}$ females, being heterozygous deficient for 7B2-3; 7C3-4 and 7D1-2; 7D5-6, respectively, are perfectly viable and fertile. The fact that heterozygous $Df(1)hl^{b,c}/+++$ females, deficient for region 7B8; 7D18, are produced almost in wild-type progeny-number suggests that region 7D18-19; 7E1-2 may contain a vital gene(s) for pupal development. Also, the fact that these females are already deficiency-heterozygous for a *Minute* is very likely to make them still less viable.

### 2. *Development*

In order to see whether the deficiency heterozygotes were delayed in development, eggs from the different crosses were allowed to develop at 17°, 25°, and 29°C. The results of these experiments are shown in fig. 2, and it can be seen that deficiency heterozygotes hatch 6–8, 4, and 2–3 days later at

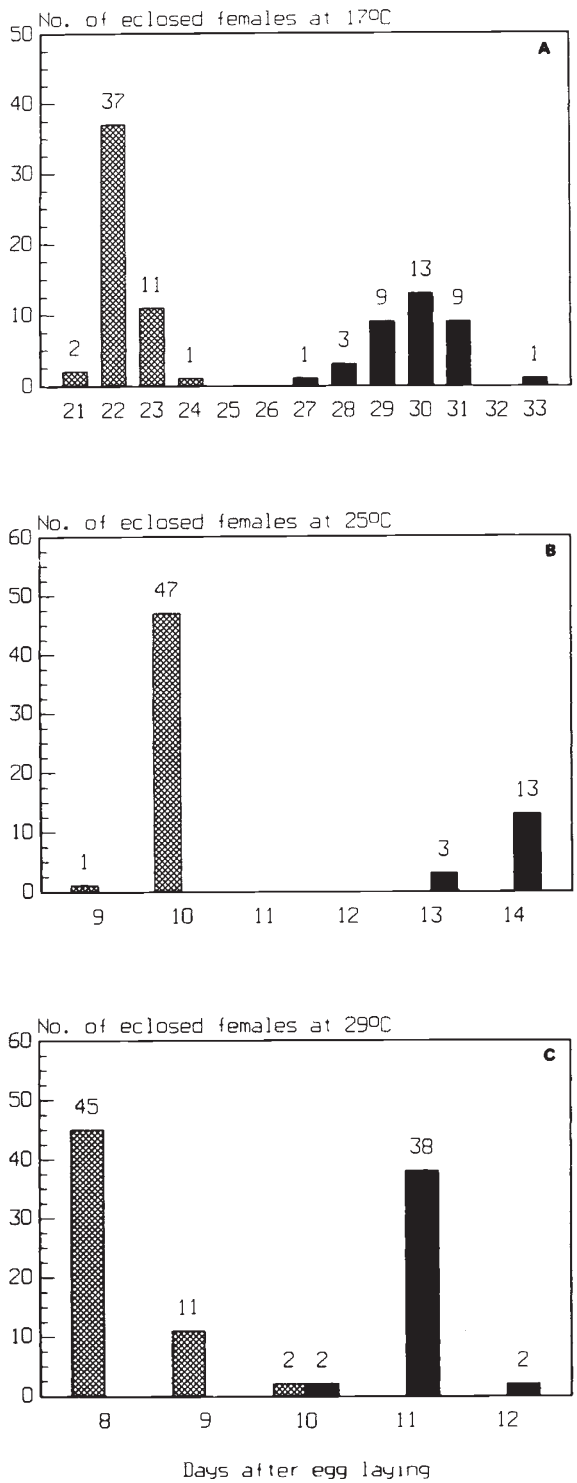


**Figure 2** Developmental time (from egg to adult) for wild-type and *Minute* females at 17°C (A), 25°C (B), and 29°C (C). Cross-hatched bars, wild-type; and filled bars, *Minute*. For further details see *Materials and Methods*.

these temperatures, respectively, in comparison with heterozygous deficient flies carrying $Dp(1;2)sn^{+72d}$. The results show that these deficiency heterozygotes have extremely retarded development, which is typical for *Minutes*. When analyzing the developmental time for the different stages we found that the delay occurred solely during the larval period.

### *3. Sterility of deficiency heterozygotes*

The fertility of *Minute* females that eclosed at the different temperatures was checked by crossing them individually to 3–5 wild-type males. If no eggs were laid after 4 days the males were replaced by males from another wild-type strain. At 25°C we found that ~50 per cent of these females were sterile, and the number of eggs laid varied from zero to that of a wild-type female. Analyzing the ovaries we found that in most cases they were normally developed, even those of females not laying any eggs. However, the common oviduct was often too narrow to allow the eggs to pass through. In some cases the ovaries appeared to be undeveloped, and the maturation time prolonged in comparison with wild-type.

The fertility of the *Minute* females was markedly reduced at 17° as well as 29°C. This result is not in accordance with the viability results, which showed a better viability at these temperatures. We did not address this problem any further.

### *4. Morphology*

When examining the deficiency heterozygotes for morphological divergence relative to wild-type we observed that they had shorter and finer bristles, rough and somewhat larger eyes, thin-textured wings with a tendency to plexus venation, missing aristae, and affected antennae (fig. 3(A, B)). It is particularly obvious that segments 4, 5, and 6 are affected, and that the number of aristae is greater on affected antennae. All the above-mentioned features are typical for *Minutes* (Lindsley and Grell, 1968). In addition, we observed flies with alterations to the morphology of the third pair of legs. On the other hand, we observed no reduction in body size, which is often seen in *Minute* heterozygotes (Lindsley and Grell, 1968).

### *5.* Minute *maternal effects*

When examining the fecundity of *Minute* females at 25°C we observed that many embryos and larvae died, and of 253 females hatched only two were

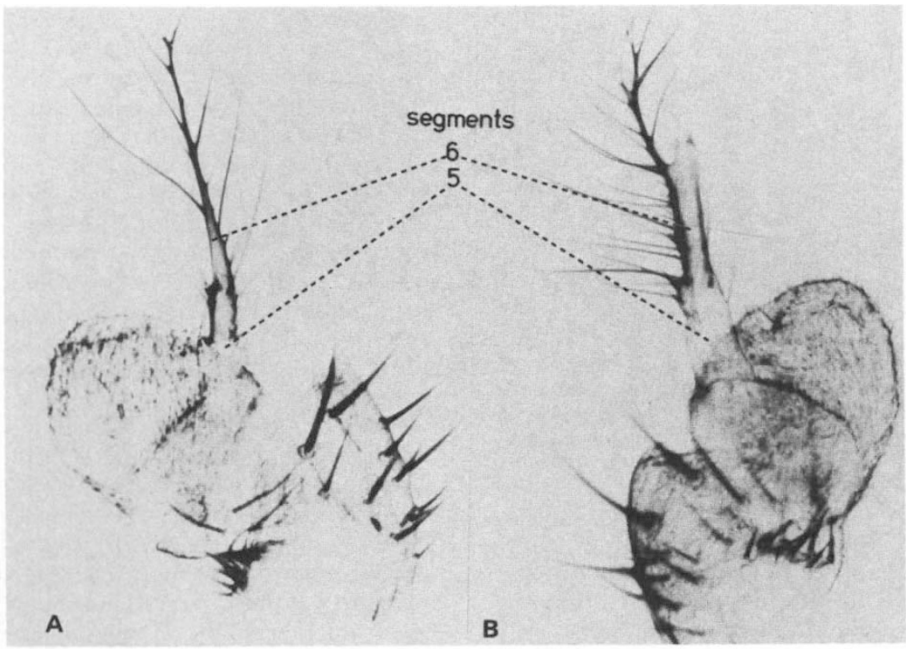


**Figure 3** Morphological effects of the *M*(1)7*C* mutation. (A) shows a normal antenna, and (B) demonstrates a typical *Minute* antenna observed in the present study.

*Minute* instead of the expected 1:1 ratio. Furthermore, both *Minute* and non-*Minute* adult progeny showed *Minute* maternal effects like abdominal segmentation defects, fused tergites, and missing or defective legs and halteres. Of the non-*Minute* offspring 30 per cent of the females ($n = 251$) and 13 per cent of the males ($n = 201$) showed these maternal effects.

*Minute* maternal effects have been observed in embryos and larvae from $M(3)i^{55}$ and *M*(3)99*D* mothers (Boring *et al.*, 1989; Kongsuwan *et al.*, 1985). Boring *et al.*, (1989) also showed that protein synthesis in embryos from *Minute* mothers is lowered by ~30 per cent during the syncytial nuclear cycles of early embryogenesis, and that the segmentation gene *fushi tarazu* (*ftz*) exhibits abnormal expression patterns at the cellular blastoderm stage. The fact that it was possible to phenocopy these *Minute* maternal effects with low doses of cycloheximide (an inhibitor of protein synthesis) supports the notion that the product of $M(3)i^{55}$ is involved in protein synthesis (Boring *et al.* 1989). Although we have no protein synthesis data on *M*(1)7*C*, the observed *Minute* maternal effects suggest that the product of this *Minute* is also involved in protein synthesis and thus indirectly may influence the expression of genes that are dependent on protein synthesis. It could be that the *RPS*14 (Brown *et al.*, 1988) and *M*(1)7*C* are the same gene.

### 6. *Northern blot analysis*

Total larval RNA from wild-type and two deficiency heterozygotes, $Df(1)hl^{b,c}$, and from wild-type ovaries was separated on agarose-formaldehyde gels, the nucleic acids were blotted onto GeneScreen Plus filters, and hybridized with pGS14-19 containing the duplicated gene for ribosomal protein *RPS*14. This gene was previously mapped to region 7C5-9 on the X-chromosome (Brown *et al.*, 1988). The autoradiogram in fig. 4 reveals that the probe hybridizes to a single transcript of ~650 b in the wild-type RNA, and that the accumulation of this transcript is extremely low in the two deficiency heterozygotes. This extremely low accumulation of *RPS*14 mRNA was a surprising observation since we expected the heterozygous deficiencies to accumulate at least 50 per cent of the wild-type amount. We have as yet no plausible explanation of this result. However, the finding implies that expression of *RPS*14 is not balanced by dosage compensation in heterozygous deficient females, and suggests that dosage com-

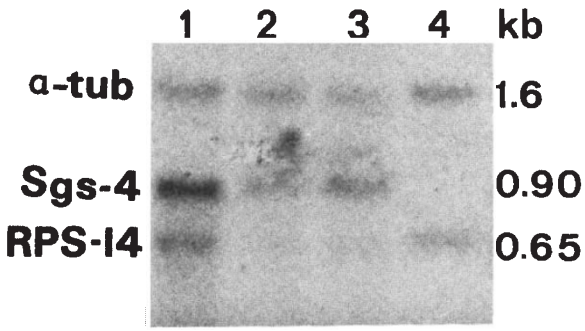


**Figure 4** Northern blot of total RNA from (1) wild-type; (2) *Df*(1)*hl*$^{b}$/+++; *bw*$^{D}$/++ (*Minute*); (3) *Df*(1)*hl*$^{c}$/+++; *bw*$^{D}$/++ (*Minute*) larvae; and (4) wild-type ovaries. 8 μg of RNA was loaded in each lane, and after electrophoresis the RNA was blotted onto GeneScreen filter. Probes pGS14-19 and α-tub were used simultaneously, and probe pRH0·75 after having deprobed the filter.

pensation mechanisms function by down-regulating female X-linked loci. The fact that the *RPS*14 gene is duplicated does not apparently solve this problem in deficiency heterozygotes (Brown *et al.*, 1988). Note that the accumulation of α-tubulin mRNA (internal standard) is not affected (fig. 4).

Probing with pRH0·75 DNA, a clone containing almost all of the *Sgs*-4 gene (salivary gland secretion), revealed that *Minute* larvae accumulate ~50 per cent *Sgs*-4 mRNA in comparison with wild-type (fig. 4). The expression of *Sgs*-4 is regulated in a temporal and spatial way (Muskavitch and Hogness, 1980; Hansson and Lambertsson 1983). The steroid hormone ecdysterone is known to be involved, indirectly as well as directly, in the control of glue gene expression (Hansson and Lambertsson, 1983, 1989). Therefore, it could be that decreased protein synthesis rates may reduce the concentrations of factors that regulate the transcription of *Sgs*-4. These factors may not necessarily be parts of the translational machinery but could be mitochondrial enzymes as has been suggested for *Minutes M*(2)*L*2 (Farnsworth, 1959) and *M*(3)*w* (Treanor, 1962; Walker, 1985). On the other hand, the observed reduction of *Sgs*-4 mRNA might be due to the *Minute* larvae not being in the same developmental stage as the wild-type ones. The tissue-specific expression of *Sgs*-4 is seen in fig. 4, lane 4, which contains wild-type ovarian RNA.

By using the pGS14-19 clone we were able to localize unambiguously the *RPS*14 gene in the X-chromosome. This was done by Southern analysis of a collection of 9 genomic lambda phage clones covering the region from 7C3-4 (*dec*-1; defective chorion-1) to 7D1-2 (*sn*; *singed*) using pGS14-19 as probe (results not shown). The clones were from a genomic lambda phage library of the *D. melanogaster* Shahrinau wild-type strain (A. Lambertsson, T. Johansson, and S. Andersson, unpublished results). We found that pGS14-19 hybridized to a clone ~28 kbp distal to clone sn9 (fig. 5). The latter clone contains the transcription startpoint of the *singed* locus, and was used as start probe in our chromosome walk to *dec*-1 (Roiha *et al.*, 1988; K. O'Hare, personal communication; A. Lambertsson , T. Johansson, and S. Andersson, unpublished results). This result maps the *RPS*14 gene to region 7C8-9, which is in agreement with the cytological mapping to 7C5-9 reported by Brown *et al.* (1988).

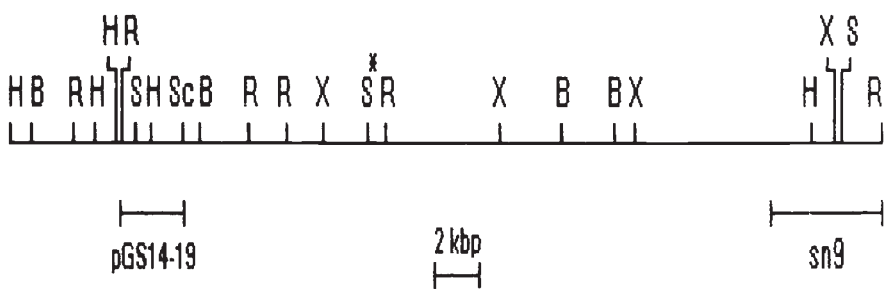


**Figure 5** Restriction map of the region containing clones pGS14-19 and sn9. The distance between the two is ~28 kbp; pGS14-19 and sn9 contain the duplicated *RPS*14 gene and the transcription startpoint of the *singed* locus, respectively. The distal to proximal orientation in the X-chromosome is from left to right (cf. fig. 1). B = *Bam*HI, H = *Hin*dIII, R = *Eco*RI, S = *Sal*I, Sc = *Sac*I, and X = *Xho*I. The asterisked *Sal*I site was not present in our Canton S wild-type stock although it was reported present in the Canton S strain used by Brown *et al.*, 1988.

## CONCLUSION

We have identified a novel *Minute* locus, *M*(1)7*C*, on the X-chromosome of *D. melanogaster*. It has most, if not all, of the *Minute* features. We have also mapped the ribosomal protein gene *RPS*14 to ~28 kbp distal from the *singed* locus; on the cytological map this is in region 7C8-9. The fact that *M*(1)7*C* and *RPS*14 both map to region 7C5-9, and that the accumulation of *RPS*14 mRNA is extremely low in two deficiency heterozygotes (*Minutes*) makes it tempting to speculate that *M*(1)7*C* and *RPS*14 are the same gene.

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