The *abnormal oocyte* Phenotype Is Correlated With the Presence of *blood* Transposon in *Drosophila melanogaster*

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ABSTRACT

The *abnormal oocyte* mutation (2;44) originates in the wild: it confers no visible phenotype on homozygous *abo* males or females, but homozygous *abo* females produce defective eggs and the probability of their developing into adults is much lower than that of heterozygous sister females. We isolated by chromosome walking 200 kb of DNA from region 32. This paper reports that a restriction enzyme site polymorphism analysis in *wild type* and mutant stocks allowed us to identify a DNA rearrangement present only in stocks carrying the *abo* mutation. The rearrangement is caused by a DNA insert on the *abo* chromosome in region 32E which, by restriction map and sequence analysis, was identified as copia-like *blood* transposon. The transposon, in strains that had remained in *abo* homozygous conditions for several generations and had lost the *abo* maternal-effect, was no longer present in region 32E. Certain features of the *abo* mutation, discussed in the light of this finding, may be ascribed to the nature of the particular allele studied.

WE focused our attention on the euchromatic region of the left arm of *Drosophila melanogaster* chromosome *2* contained within region 32 of the standard salivary gland chromosome map and defined by a set of mutants that exhibit striking similarities (SANDLER 1977). Each of these mutants, *hup*, *wdl*, *dal* and *abo*, is recessive, hypomorphic, maternal-effect and female semisterile. Of the four mutants, *abo* is the best characterized and the only one that originates in the wild (isolated in 1965 by L. SANDLER in the outskirts of Rome); the others are EMS-induced mutants isolated by SANDLER (1977) in an attempt to identify other *abo* alleles. The *abnormal oocyte* mutation (2;44) confers no visible phenotype on homozygous *abo* males or females, but homozygous *abo* females produce defective eggs and the probability of their developing into adults is much lower than that of heterozygous sister females and can be augmented by increasing the dose of particular heterochromatic regions in the mother and/or in the zygote (SANDLER 1970; PARRY and SANDLER 1974; PIMPINELLI *et al.* 1985).

Because of this interaction with heterochromatin, *abo/abo* females carrying *wild-type X* chromosomes, when crossed to attached *XY*/O; abo^{+}/abo^{+} males, produce an excessive number of female offspring as a result of increased embryonic lethality of the *X*/O relative to the $X/\overline{XY}$ zygotes produced (SANDLER 1970).

By a genetic approach MALVA *et al.* (1985) have demonstrated that there is a maternal and zygotic interaction between the *abo* gene located on the second chromosome and the specific heterochromatic region of the sex chromosome, identified by the proximal breakpoint of the sc^{4} inversion.

It has been reported (KRIDER and LEVINE 1975) that in homozygous *abo* stocks the *abo* maternal-effect gradually decreases, as measured by the sex ratio in crosses to attached *XY*/O males, and is lost after several generations in homozygous conditions (*abo*Gn). It has been thought that the heterochromatic segments interacting with *abo* influence in turn the structural cistrons for ribosomal RNA located within the sex heterochromatin (RITOSSA, ATWOOD and SPIEGELMAN 1966) because, during the loss of the *abo* phenotype, the rDNA content of each *X* chromosome increases (KRIDER and LEVINE, 1975; KRIDER, YEDVOBNICK and LEVINE 1979; YEDVOBNICK, KRIDER and LEVINE 1980). In addition, the rDNA increase has been associated with variations in the restriction pattern of the non-transcribed spacer (GRAZIANI *et al.* 1981). MANZI *et al.* (1986) have reported that loss of the *abo* phenotype can also occur without an rDNA increase and that, therefore, this phenomenon may be due to other events occurring in other regions of sex chromosomes, possibly the heterochromatic regions.

We performed a restriction enzyme site polymorphism analysis of 200 kb of chromosome walking in region 32 in *wild type* and mutant stocks and identified,

In memory of LARRY SANDLER.

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with the recombinant phage called E3 and mapping in 32E, a DNA rearrangement present only in stocks carrying the *abo* mutation. In this paper we report the identification, isolation and characterization of the region responsible for the difference between the *wild-type* and *abo* restriction patterns and demonstrate the presence of a DNA insert, about 7.2 kb long, on the *abo* chromosome in region 32, identified by phage E3. By restriction map and sequence analysis, we found that the element is the copia-like *blood* transposon first identified by BINGHAM and CHAPMAN (1986) in the *white blood* (w^{bl}) mutation (EPHRUSSI and HEROLD 1945).

Interestingly, in specific genetic conditions where the *abo* maternal-effect is lost, *i.e.*, in strains that have remained in *abo* homozygous conditions for several generations, the transposon is no longer present in region 32.

This is not a direct demonstration that phage E3 contains the DNA sequence corresponding to the *abo* gene, which can be obtained by other approaches, but directly correlates changes in DNA structure with changes in phenotype and, hence, provides a new insight into the nature of the *abo* phenotype.

MATERIALS AND METHODS

***D. melanogaster* stocks:** We used *wild-type Oregon R* from EMBL (Heidelberg), IIGB (Naples) and the University of Naples; *Canton S* from IIGB (Naples).

The *abo/Cy*, *dal/Cy*, *wdl/Cy* and *hup/Cy* stocks came from SANDLER's laboratory, University of Washington, Seattle, Washington. A recombinant *dp cl abo* chromosome was constructed in our laboratory as follows: homozygous *dp cl b* females were crossed with *abo/abo* males; the female progeny were then crossed with *dp cl b* males and the recombinant *dp cl* progeny were collected and cloned with a *Cy* balancer. The recombinant chromosomes were then singly tested for the presence of the *abo* mutation. For further details on the markers used see LINDSLEY and GRELL (1968).

For the collection of third instar larvae the *wild-type* stock was raised at 18° or at 22° on a standard sucrose-corn meal-yeast medium.

Nucleic acid preparation: Genomic DNA from adult flies was extracted by standard procedure (ENDOW and GLOVER 1979). Phages and plasmid DNA were extracted according to MANIATIS, FRITSCH and SAMBROOK (1982).

Libraries and subclones: A genomic library (kindly provided by V. PIRROTTA) was constructed by partial *Sau*3A digestion of *Oregon R* genomic DNA and ligation into the *Bam*HI site of the EMBL4 vector (FRISCHAUF *et al.* 1983). Average length of the inserts was about 18 kb. An adult female cDNA library, provided by T. KORNBERG, was in λgt10 (POOLE *et al.* 1985).

Screening of genomic and cDNA libraries was performed by *in situ* plaque hybridization (BENTON and DAVIS 1977). The cDNA library was screened according to standard procedure (MANIATIS, FRITSCH and SAMBROOK 1982).

Subclones were generated by insertion of restriction fragments of the clones (lambda or plasmid) into appropriate site(s) of the pEMBL8 or puC19 vectors.

Restriction analysis, electrophoresis, transfer and hybridization of DNA: Digestions with restriction enzymes were carried out under conditions recommended by the manufacturers (Amersham, Biolabs, Boeringher). Restriction maps were determined by analyzing single and double digestions on 0.4–1.5% agarose gels in Tris-borate EDTA buffer (MANIATIS, FRITSCH and SAMBROOK 1982). The DNA was transferred onto nitrocellulose filters, hybridized as outlined by SOUTHERN (1975) and washed two times in 2 × SSC, 0.1% SDS for 30 min at 65° and four times in 0.1 × SSC, 0.1% SDS for 30 min at 65°.

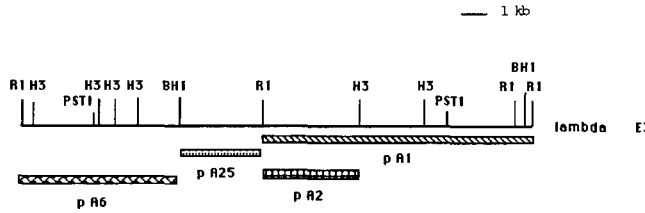

FIGURE 1.—Restriction map of the lambda phage E3 and of its four Puc19 subclones.

***In situ* hybridization:** *In situ* hybridization to the polytene chromosomes was carried out according to PARDUE and GALL (1975). For the *in situ* hybridization, probes were labeled by nick-translation (RIGBY *et al.* 1977) with [^{3}H] dTTP or [^{3}H]dCTP (specific activity 0.5–1 × 10^7 cpm/μg).

Preparation and screening of the mini-library: An aliquot of 50 μg of genomic DNA from *abo/abo* females was digested with *Eco*RI/*Hin*dIII and 4.6-kb long fragments were recovered from preparative gels by means of electroelution. The recovered DNA was ligated into *Eco*RI digested λgt10 arms after defosforylation. After packaging with Stratagene extracts the recombinant clones were screened according to standard procedure (MANIATIS, FRITSCH and SAMBROOK 1982).

DNA sequencing: The nucleotide sequence was determined by using the subclones in pUC9, pEMBL8 and pUC19 directly by the dideoxynucleotide chain termination method of HATTORI, HIDAKA and SAKAKI (1985).

RESULTS

Restriction site polymorphism analysis: For a better understanding of the *abo* and other maternal-effect gene functions in region 32 and for an elucidation of the molecular mechanisms involved in the loss of the *abo* phenotype, we decided to isolate these genes. Starting with plasmid clone 153B9 (kindly provided by WALTER GEHRING) containing a *D. melanogaster* segment from region 32D we screened a Drosophila library constructed in the EMBL4 lambda phage vector with embryonic DNA from the *Oregon R* stock and walked about 200 kb in this region, from band 32D to band 32E-F. We used the phages we had previously isolated for various kinds of analyses (GIGLIOTTI *et al.* 1989). We also used these phages to identify restriction enzyme site polymorphisms in this region and possibly correlate them with the mutations we were studying. DNAs from homozygous or heterozygous mutant *abo*, *dal*, *wdl* and *hup* flies and from *Canton S* and *Oregon R wild-type* stocks were digested with restriction enzymes and probed with the single isolated

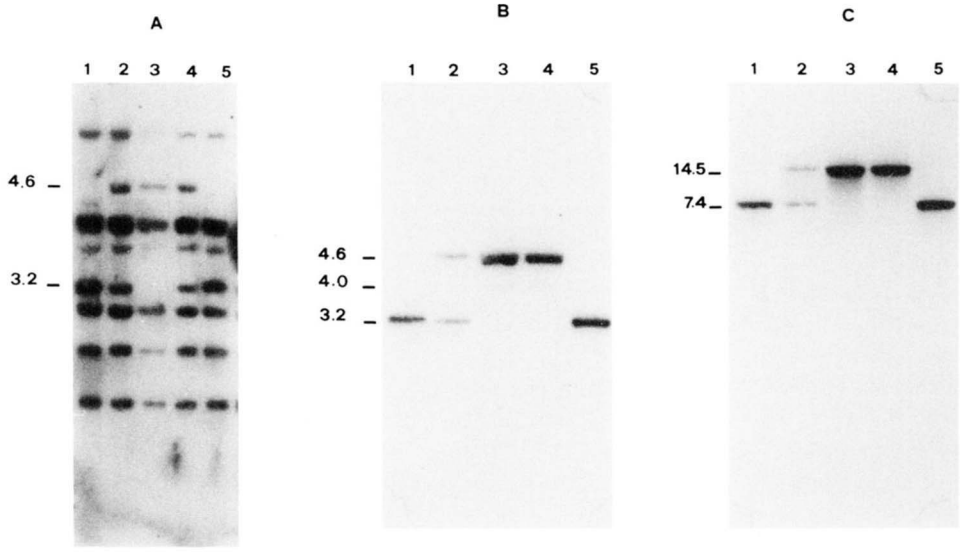

FIGURE 2.—Southern blot analysis of: (A) genomic DNA from *Canton S* (1), *abo/Cy* (2), *abo/abo* (3), *abo/Cy* (4), *dal/Cy* (5) females, digested with *Hin*dIII/*Eco*RI and hybridized with phage E3 as probe. The arrow indicates the 4.6 and 3.2 bands. (B) genomic DNA from *wild type* (1), *abo/Cy* (2), *abo/abo* (3 and 4) and *abo/abo*Gn (5) females, digested with *Hin*dIII/*Eco*RI and (C) genomic DNA from *wild type* (1), *abo/Cy* (2), *abo/abo* (3 and 4) and *abo/abo*Gn (5) females, digested with *Hin*dIII. The probe used in B and C was the *Hin*dIII/*Eco*RI fragment of phage E3 identified by the subclone named pA2 in Figure 1. The *Hin*dIII/*Eco*RI (B) digestions give, only in strains carrying at least one *abo* chromosome (lanes B, 2, 3 and 4), the 4.6-kb band seen in panel A, as well as a faint 4.0-kb band which is absent in *wild-type* stocks.The 3.2-kb fragment is present only in strains carrying at least a *wild-type* chromosome. With *Hin*dIII the *wild-type* DNA (lane C, 1) gives a 7.4-kb fragment, while the *abo* DNA (lanes C, 2, 3 and 4) gives a heavier 14.5-kb fragment. The restriction pattern of the *abo/abo*Gn line (lanes 5, B and C), in homozygous conditions for several generations and having an abo^+ phenotype, is identical to that of *wild type* with the two restriction enzymes used.

phages. We found several restriction site polymorphisms in the region but only one associated with only one mutation. Using phage E3 (Figure 1) as probe, only when an *abo* chromosome was present in the stocks used to extract and digest genomic DNA with *Hin*dIII/*Eco*RI (Figure 2A, lanes 2, 3 and 4) did we observe the presence of a 4.6 kb band, absent in all the other stocks. Moreover, only in *abo* homozygous flies did a 3.2 kb band, present in all the stocks, disappear (Figure 2A, lane 3).

Polymorphism is due to a DNA insertion: Given the relative complexity of the restriction pattern obtained with whole phage E3, we used smaller fragments or subclones (Figure 1) to identify the smallest fragment responsible for the polymorphism and to obtain more information on the kind of rearrangement present on the *abo* chromosome.

We found that the differences were limited to the *Hin*dIII/*Eco*RI fragment of phage E3 identified by subclone pA2 in Figure 1. In fact, using this subclone as probe in Southern blot analysis on genomic DNA digested with *Hin*dIII/*Eco*RI (Figure 2B), we observed, only in stocks carrying at least one *abo* chromosome (lanes 2, 3 and 4), the 4.6 kb band seen in the first experiment as well as a faint 4.0-kb band which was absent in abo^+ stocks. On the other hand, the 3.2-kb fragment was present only in stocks carrying at least one abo^+ chromosome (lanes 1 and 2).

With *Hin*dIII, the *wild-type* DNA (Figure 2C, lane 1) gave a 7.4 kb fragment, like that of phage E3, while the *abo* DNA (Figure 2C, lanes 3 and 4) gave a heavier 14.5-kb fragment.

In all the restriction site polymorphism analysis experiments, a systematic criterion in the attempt to correlate a DNA rearrangement with a mutant phenotype was to investigate the molecular structure of the DNA extracted from one of the strains in our stock collection, which had lost the *abo* phenotype after remaining in homozygous conditions for several generations (*abo*Gn). In lane 5 of Figure 2, B and C, such a strain clearly shows the molecular organization present in *wild-type* stocks, with the two restriction enzymes used.

The Southern analysis indicates that we are not in the presence of a base change that causes a specific restriction site polymorphism in the *abo* strain but that on the chromosome carrying the *abo* mutation in region 32E a DNA fragment about 7.2 kb long is inserted in the *wild-type* genomic region identified by the pA2 clone. The inserted fragment contains at

least one *Eco*RI and no *Hin*dIII sites. Obviously due to the use of a *wild-type* probe, the fragments inside the DNA insertion are not visible in these experiments. The minor intensity of the 4-kb *Eco*RI fragment seen in lanes 3 and 4 of Figure 2B may be explained by an asymmetrical insertion of the DNA fragment into the *wild-type* pA2 region and, therefore, to a limited homology between genomic DNA from the *abo* mutant strain and the *wild-type* probe.

DNA insertion is a middle repetitive element: the *blood* retrotransposon: Given the strong correlation between the rearrangement identified in region 32 and the *abo* phenotype, we identified the DNA insertion present in the *abo* chromosome by constructing a partial *Hin*dIII/*Eco*RI library of genomic DNA from the homozygous *abo* stock in the λgt10 vector and by isolating clones that hybridize to the pA2 clone. Figure 3 shows the restriction map of the 4.6 clone isolated from the *abo* genome compared to the corresponding *wild type* pA2 clone. The two clones are identical for 2.7 kb, containing *Eco*RI, *Hin*dII and *Sal*I sites. After this last site the map is completely different, confirming that a different sequence is present in region 32 of the chromosome carrying the *abo* mutation.

We sequenced (Figure 3) the 423 bp *Xba*I-*Sal*I fragment of the *wild type* pA2 clone and the 516 bp *Sal*I-*Sal*I fragment from the 4.6 clone which, from the restriction maps, should contain one of the junctions between the *wild-type* sequence and the DNA insertion. As expected, after the first 291 nucleotides (nt) the sequences totally diverged to the end of the respective fragments. A computer-assisted analysis of the sequence from nt 292 to nt 516 of the 4.6 clone with the available DNA sequence banks gave limited homologies with the LTR sequences of certain copia-like elements contained in the Bank. However, a direct inspection of the LTR sequences of the copia-like elements about 7 kb in length reported by FINNEGAN and FAWCET (1986) allowed us to identify an almost 100% similarity between our 225 nt sequence and the LTR of the *blood* transposon identified by BINGHAM and CHAPMAN (1986) in the *white blood* mutation (EPHRUSSI and HEROLD 1945). This transposon was 7.4 kb long, had a *Sal*I site in its LTR and its restriction map was identical to the restriction map of that region of the 4.6 clone corresponding to the DNA insert we characterized in the *abo* strains. As a further control experiment, with the enzyme *Sal*I we digested genomic DNA from *wild type*, *abo/abo*, *abo/Cy* and *abo/abo*Gn strains, Southern-blotted and hybridized with a subclone containing only the repeated segment of the 4.6 clone (Figure 4). As expected, a 7.0-kb band appeared in all the strains.

We also analyzed the presence and distribution of *blood* transposon by Southern blots and *in situ* hybrid-

GTCGACTGACGGATACGATAAATATTGGATTCCTGGCTGAA
TATTCTCAGATGCGAGTGAGTGAAGCGTGGAGATCGGGCAA
CTGCTAAGACGGTTTTAAATTTGTGAAAAATTATCGAAATT
AATTGTGTATTTCGTCCTGTGGTAATTGTGTATGTGCATGC
ATAATTCATCGACCTGTCACATTTGCAATTCAAATGGCTAT
CTCATTTTGGAGAGCACAAGTCAACGCATCCTTTCCCAAAA
ATATGCCAACATTTATGGCTCAGCACGATGATTGAAGGGAC
TAGTCTGTTGCCCTAATGAAACAAATCATGCGTCCCTTGGG
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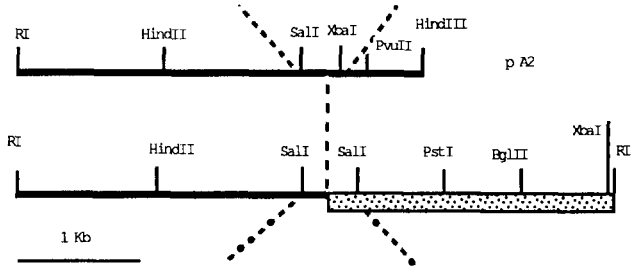

GTCGACTGACGGATACGATAAATATTGGATTCCTGGCTGAA
TATTCTCAGATGCGAGTGAGTGAAGCGTGGAGATCGGGCAA
CTGCTAAGACGGTTTTAAATTTGTGAAAAATTATCGAAATT
AATTGTGTATTTCGTCCTGTGGTAATTGTGTATGTGCATGC
ATAATTCATCGACCTGTCACATTTGCAATTCAAATGGCTAT
CTCATTTTGGAGAGCACAAGTCAACGCATCCTTTCCCAAAA
ATATGCCAACATTTATGGCTCAGCACGATGATTGAAGGGAC
TAGTGTAGTATGTGCATATATGCAGGTACACTGTACCTATA
AGTACACAGCAACACTTAGTTGCATTGCATAAATAAATGTC
TCAAGTGAGCGTGGATAATAAGATCACCCATTTATGCTTTA
AGCTAAGTCAGCTACCCCACGCGCCGCTGGCCATATATGCG
CATAAGCTCTCTCTCTCTCTCTCTTATACATATATATATAC
GCTGCTCTTCTGCCGCTGGTCGAC

FIGURE 3.—Restriction map of the 4.6 clone isolated from the *abo* genome compared to the corresponding *wild-type* pA2 segment and sequence of the corresponding 423 bp *Xba*I-*Sal*I fragment and 516 bp *Sal*I-*Sal*I fragment The two clones are identical for 2.7 kb until the *Sal*I sites. After this last site, the maps and sequences are completely different.

ization. We used *Pst*I, *Sac*I and *Eco*RI enzymes which, according to BINGHAM and CHAPMAN (1986) as well as our restriction maps, have only one site in the region corresponding to the 2.1 terminal part of the *blood* transposon used as probe. Figure 5 reports the pattern obtained using genomic DNA from Xsc^4/Xsc^4; *abo/abo* X^+/X^+; *abo/abo*Gn and X^+/X^+; *abo/abo* females, digested with *Pst*I (lanes 1, 2 and 3), *Sac*I (lanes 4, 5 and 6) and *Eco*RI (lanes 7, 8 and 9), respectively. With *Pst*I and *Sac*I we obtained several bands, as expected. Surprisingly, with *Eco*RI we obtained only a major band of 1.8 kb, indicating that most of the elements have an additional *Eco*RI site after the LTR excepting the one localized in region 32. In fact, Figure 5 shows that with this enzyme the 4.6-kb band

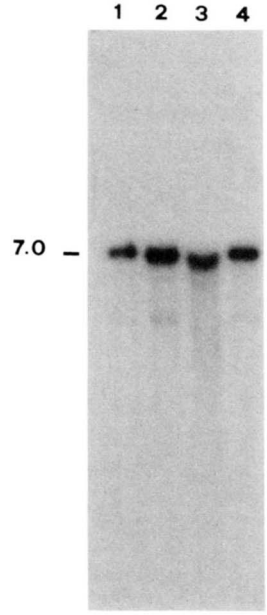

FIGURE 4.—Southern blot of genomic DNA from *wild type* (1), *abo/abo* (2), *abo/Cy* (3) and *abo/abo*Gn (4) females, digested with the *Sal*I enzyme and hybridized with a subclone containing only the repeated region of the 4.6 clone. A 7.0-kb band is present in all the strains analyzed.

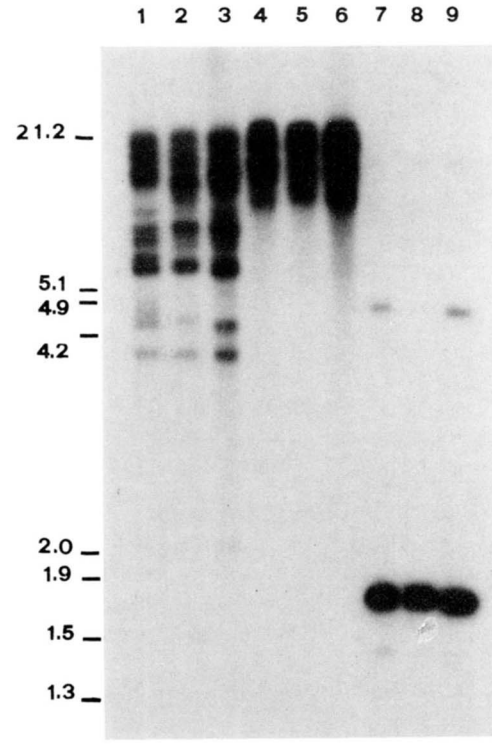

FIGURE 5.—Southern blot analysis of genomic DNA from Xsc^4/Xsc^4; *abo/abo*, X^+/X^+; *abo/abo*Gn and X^+/X^+; *abo/abo* females, digested with *Pst*I (lanes 1, 2 and 3), *Sac*I (lanes 4, 5 and 6) and *Eco*RI (lanes 7, 8 and 9) and hybridized with the subclone containing only the repeated region of the 4.6 clone, the same used in Figure 5.

appears in the two *abo* strains (lanes 7 and 9), absent in the *abo*Gn strain (lane 8), which hybridizes also with the pA2 subclone from region 32 (data not shown). The same peculiarity, namely the lack of this *Eco*RI site, seems to be shared by the *blood* element isolated by BINGHAM and CHAPMAN (1986): we wonder if this structural difference is correlated with the ability of transposing and, therefore, causing new mutations. Among the strains analyzed in Figure 5, was the *abo/Cy* strain carrying the sc^4 inversion on the *X* chromosomes In this strain the *abo* maternal-effect is so strong that it is impossible to obtain any progeny in crosses of homozygous sc^4 and *abo* flies (MALVA *et al.* 1985). The restriction pattern obtained on genomic DNA from Xsc^4; *abo/abo* females (lanes 1, 4 and 7) presents interesting differences, which we intend to analyze further.

By *in situ* hybridization (Figure 6), several localizations of *blood* were found scattered along all the chromosomes, as expected. A more detailed analysis is in progress to compare the *blood* localizations in the different genetic conditions of the *abo* strains.We prepared a genomic library from homozygous *abo* flies and isolated the complete *blood* transposable elements. We are in the process of characterizing the isolated clones in order to sequence the complete transposon present on the *abo* chromosome.

***blood* transposon is lost together with the *abo* phenotype:** From the beginning of our study on the correlation between loss of the *abo* phenotype and amount of rDNA, we started to build a collection of different independent *abo* homozygous lines. All these lines, which have been in homozygous conditions for as many as 170 generations (Gl70), today no longer have the *abo* maternal-effect as measured by the sex ratio in the progeny of the *abo* diagnostic crosses between *abo* homozygous females and attached *XY*/O males. The phenotype of these lines is, therefore, *wild type*. To be sure that with the loss of the abo phenotype we were and are always following the original *abo* chromosome, sometime ago we constructed a stock in which, by recombination, the *dp cl* markers were transferred to the original *abo* chromosome (see MATERIALS AND METHODS). In this new *dp cl abo* recombinant chromosome, the DNA rearrangement described was transferred together with the *abo* mutation. We used this recombinant chromosome to construct other homozygous lines.

Given the interesting results obtained with one of the *abo/abo*Gn strains used in the restriction site polymorphism analysis, where the molecular structure of region 32E was identical to that in the *wild-type* stocks, we decided to screen other lines that had been kept in homozygous conditions for so many generations that they had lost the *abo* phenotype, using as control the original heterozygous *abo/Cy* stock. After digesting the DNA with different restriction enzymes and using as probe the pA2 clone in Southern blots, we observed the molecular organization present in the *wild-type* stock in all these lines and with all the restric-

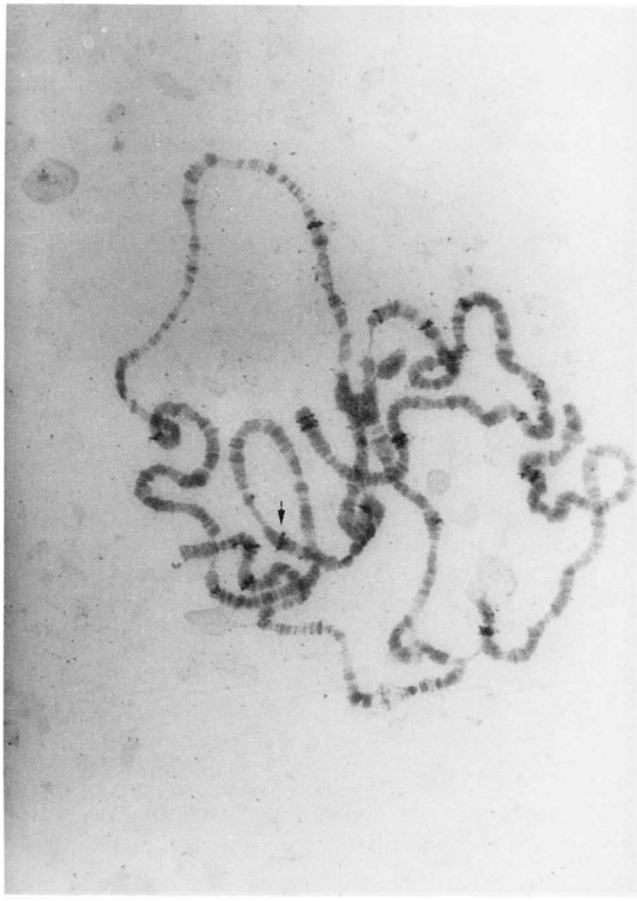

FIGURE 6.—*In situ* hybridization of the 4.6 clone on the salivary gland polytene chromosomes of *Canton S* stock. The 4.6 fragment used as probe contains a unique sequence from region 32 indicated by the arrow and a repeated fragment with several localizations along all the chromosomes.

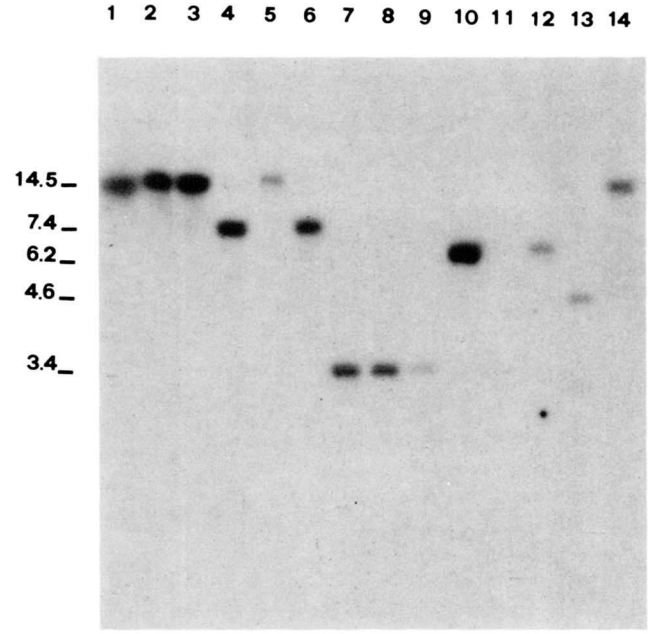

FIGURE 7.—Southern blot analysis of genomic DNA from *abo/abo* females of different strains showing the following phenotypes: *abo* (1), *abo* (2), *abo* (3), abo^+ (4), *abo* (5), abo^+ (6), digested with *Hin*dIII; *abo* (7), *abo* (8), *abo* (9), abo^- (10), *abo* (11), abo^+ (12), digested with *Pst*I/*Eco*RI; *abo* (13), digested with *Hin*dIII/*Eco*RI; and *abo* (14), digested with *Hin*dIII. The probe used is the pA2 clone. Lanes 1, 2, 7 show the stock in which, by recombination, the *dp cl* markers were transferred to the original *abo* chromosome. Also in this new *dp cl abo* recombinant chromosome, the DNA rearrangement is present and the loss of the *abo* phenotype in *abo* homozygous strains (lanes 6 and 12) is associated with the loss of the transposon.

tion enzymes used. Some of these lines are shown in Figure 7. These lines were all indistinguishable from *wild type* although they differed greatly from each other because each one had a different number of generations in *abo* homozygous conditions. In the hope of documenting at a molecular level the loss of the *abo* phenotype, we decided to start a new homozygous line, henceforth called line A, and to follow generation by generation (1) the *abo* phenotype by genetic approaches (number of progeny per mother, diagnostic cross, etc.) and (2) the molecular structure revealed by the pA2 clone. The experiment, schematized in Figure 8, was performed as follows: from the heterozygous *abo/Cy* stock, homozygous virgin females, which we named the G_0 generation, were used in the diagnostic cross to test the *abo* phenotype. At the same time other homozygous virgin females and homozygous males were used to start the homozygous line. After one week of ovodeposition, the founder population was kept at −80° for molecular analysis. The progeny of this cross, representing the G_1 *abo* generation, were used for the same period of time (10 days) to test the abo phenotype and to give rise to the G_2 generation. At each successive generation some of the flies were kept at −80° for molecular analysis and some were used to test the *abo* phenotype. In this experiment we observed a persistence of the *abo* phenotype up to the sixth generation and a sudden disappearance of the maternal-effect in the seventh generation. In previous experiments a more gradual appearance of abo^+ flies had been observed. The pattern observed in Southern analysis performed with *Eco*RI/*Hin*dIII digestions shows that the appearance of the abo^+ phenotype was accompanied by the appearance of a *wild-type* band. In the first generations in *abo* homozygous conditions, the progeny were in very small number due to the semisterility of the *abo/abo* mothers. Some of the progeny flies were used to start the successive generations and some to test the *abo* phenotype. In these conditions, certain discrepancies could be found between the two small populations and, therefore, between the molecular and genetic analyses. This may explain the fact that in the sixth generation the phenotype was still *abo* but the *wild-type* band had already appeared. This result is important because it reinforces the correlation between the presence of the blood transposon and the *abo* mutation. In addition it shows the rapid loss of a copia-like element during the propagation of a line, which was never observed before and that we could probably

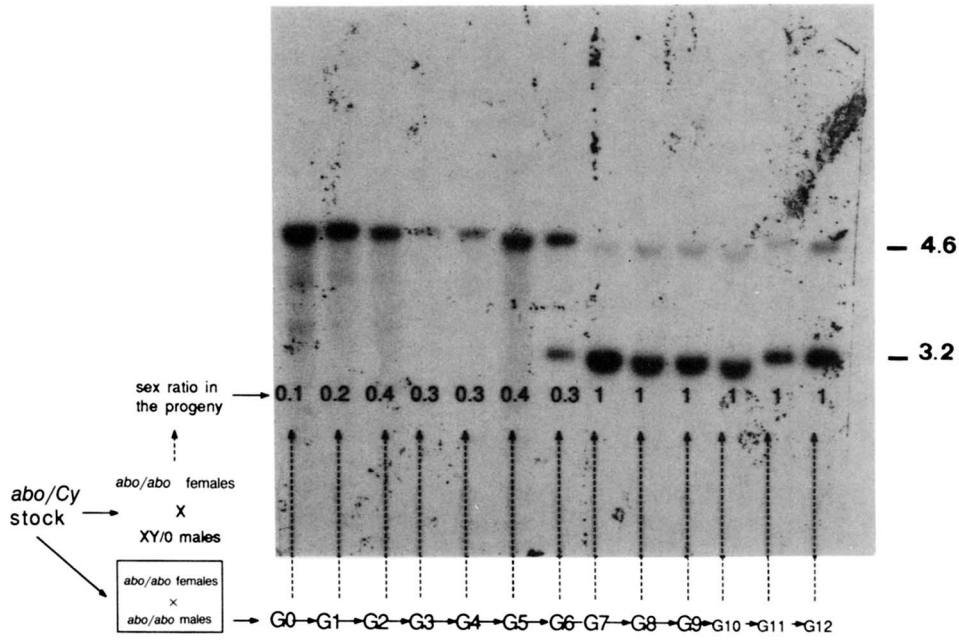

FIGURE 8.— Southern blot analysis of genomic DNA from strain A females of different generations in homozygous conditions, digested with *Hin*dIII/*Eco*RI and hybridized with the pA2 clone. The scheme indicates the number of generations and the sex ratio obtained in the *abo* diagnostic cross for each generation (for details see the text). Sex ratio is measured as the ratio of males to females in the experiment since in control experiments sex ratio is close to 1.

detect because the loss of the transposon has been selected for. We therefore decided to repeat the experiment using a line (line B) with a different genetic background and also we started with a greater number of flies and used for the molecular analysis also the flies used for the diagnostic test. Figure 9 reports the result obtained with line B which fully confirms the result of the previous experiment: in this homozygous *abo* line, at generation 9, where the sex ratio change to 0.9, the molecular pattern became *wild type*. In this experiment, even if the number of flies was greater, molecular and genetic tests were not performed at generation *abo* 2, 7 and 8 because of problems in the propagation of the strain. In this case the discrepancy observed in the experiment of Figure 8, where at generation 6 the flies were still *abo* but the wild type band already appeared, seems no longer observed because at generation 9 where the *wild-type* band appears the phenotype is abo^+: unfortunately we do not have the information on generation 8.

From the homozygous *abo8* generation of line A, where the loss of the *abo* phenotype was observed, we have constructed heterozygous *abo/Cy* lines, at present at the 15th generation in heterozygous conditions: we have not yet observed the reappearance of the *abo* phenotype in homozygous females from this heterozygous strain.

We are preparing a genomic library from the reverted line to investigate, by sequencing, the structure of the reverted site, which, on the basis of Southern analysis, is indistinguishable from *wild type*.

DISCUSSION

In this paper we report the identification of a polymorphism due to the insertion in region 32E of the *abo* stocks of the copia-like *blood* transposon, first identified by BINGHAM and CHAPMAN (1986) in the *white blood* mutation. In addition, we demonstrate a strict correlation between the presence of *blood* transposon and the *abo* phenotypic expression. Whereas the *abo* stock contains this element in region 32E, the *wild type* and certain *abo* homozygous strains, which have lost the *abo* maternal-effect, do not. This paper also represents the first report of the loss of a retrotransposon occurring at a rate which implies that the spontaneous excision frequency of this particular *blood* transposon must be rather high, even though the loss of the transposon is being selected for.

Obviously these data did not lead to the precise identification of the sequences corresponding to the *abo* gene. We are studying the transcripts identified by genomic and cDNA clones in region 32E and looking for differences in the RNA profile between the *wild type* and *abo* stocks. Given the temperature sensitivity of the *white blood* and *abo* mutants, we are also studying RNA transcription and *abo* phenotype at different temperatures. The features emerging are complex and at the moment our data do not seem to suggest a simple correlation between changes in the RNA pattern and the *abo* phenotype.

Several features of the behavior of the *abo* mutation, documented in various reports (KRIDER and LEVINE 1975; SANDLER 1975; GRAZIANI *et al.* 1981; MANZI *et al.* 1986), have been difficult to explain in a unitary model: the instability of the *abo* phenotype, the influence of heterochromatin, the irreproducibility of certain data such as the ribosomal DNA increase in *abo* homozygous lines. Even in this paper, with regard to the irreversibility of the loss of the *abo* phenotype, we are confronted with results contrasting with those

B	*abo1*	*abo2*	*abo3*	*abo4*	*abo5*	*abo6*	*abo7*	*abo8*	*abo9*	*abo10*	*abo11*
♂♂ / ♀♀	0.1	/	0.2	0.3	0.2	0.1	/	/	0.96	1	0.99
total progeny	157	/	251	453	370	150	/	/	560	798	692
progeny per mother	7	/	10	11	19	9	/	/	28	40	47.2

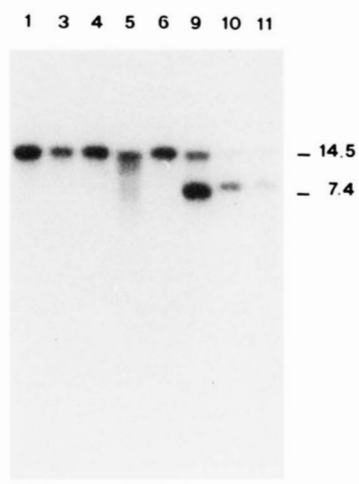

FIGURE 9.—The same experiment illustrated in Figure 8 except that DNA was digested with *Hin*dIII and total progeny and progeny per mother are given for all the generations in *abo* homozygous condition examined.

previously reported. In fact, as far as we can say at present, the loss of the *abo* phenotype seems irreversible at least in the strains we analyzed. The data reported in this paper, however, describe for the first time a molecular feature of the *abo* chromosome that may explain most of these phenomena.

KRIDER and LEVINE (1975) have observed the recovery of the *abo* mutation from strains that had lost the abo phenotype. The obvious explanation was that the loss of the *abo* phenotype was not due to a selection of revertant abo^+ flies. The results reported in this paper, where we had for the first time the opportunity to analyze region 32 of the different strains at a molecular level, seem to indicate that a reversion to the *wild-type* DNA structure occurs and, therefore, that recoyery of the *abo* mutations from the reverted strains seems unlikely. It seems clear at this point that the reversion of the *abo* phenotype can occur by several different pathways. At least in the homozygous strains analyzed in this paper we can hypothesize that the loss of *blood* transposon was the favored mechanism of reversion to *wild type.* This may be due either to chance or to the genetic background of line A presenting an unknown increased capacity of excision of the transposon (possibly explained by the unexpected relatively low number of generations required for the loss of the *abo* phenotype). The excision of the transposon confers such a selective advantage in homozygous conditions that the flies in which the event occurs completely substitute the *abo* flies. As for the other lines, the apparent permanent loss of the transposon and of the *abo* phenotype may not correspond to the phenomena that had occurred in the past considering the fact that the lines had been in homozygous conditions for an enormous number of generations, as many as 170. Whether the transposon is lost in the heterozygous strains remains an open question. It is possible that in heterozygous conditions the transposon in region 32E confers some unknown selective advantage or that the loss of the transposon is highly facilitated only in homozygous conditions.

The identification of *blood* transposon in region 32E of the *abo* chromosome and the recent elucidation of the mechanism of suppression of the *gypsy*-induced mutations (MODELL, BENDER and MESELSON 1983; SPANA, HARRISON and CORCES 1988; PARKHURST *et al.* 1988; PEIFER and BENDER 1988) may help us understand the mechanisms responsible for the influence of heterochromatin on the expression of the *abo* phenotype. It has been demonstrated that *su(Hw)* encodes a putative DNA-binding protein (PARKHURST *et al.* 1988) that interacts with specific DNA sequences of the *gypsy* element (SPANA, HARRISON and CORCES 1988) to control its transcription and, as a consequence, its mutagenic effect. If in *blood* transposon as well a specific sequence interacts with a specific modifier gene product and if in heterochromatin *blood* transposon, or only its regulatory sequence, is repeated several times, the addition of heterochromatin may titrate the modifier protein thus allowing, to a certain extent, the proper expression of the abo gene altered by the presence and active transcription of the transposon in region 32E. The different degree of the *abo* maternal-effect in different genetic backgrounds (MANZI *et al.* 1985) may be due to a different heterochromatin constitution in the different strains analyzed. In addition, changes in the heterochromatin structure in the same strain after several generations may produce the loss of the *abo* phenotype in *abo* homozygous strains without requiring any change in the structure of the *abo* region, as was suggested to explain the loss of the *abo* phenotype in the experiments where the loss seemed itself reversible.

The existence of an interaction between specific sequences of *blood* transposon and a specific modifier gene suggests a third mechanism for the disappear-

ance of the *abo* phenotype. It may directly involve mutations occurring in the hypothesized modifier gene which possibly acts as suppressor of the *abo* mutation, in analogy with the *gypsy* and *su(Hw)* system.

Finally, if the loss of the *abo* phenotype can occur by several pathways that differ with regard to the possibility of recovering the original mutation, we can explain the difficulty in reproducing after several years some of our and other authors' laboratory results, such as the rDNA increase in homozygous *abo* strains. The accidental occurrence of mutations in modifier genes or changes in the structure of heterochromatin in the strains analyzed may influence the probability of going through one of the possible pathways for the loss of the *abo* phenotype. That the *blood* sequences hybridize in the chromocenter and that in the sequenced 225 nt of *blood* transposon the enhancer-like motif identified in *gypsy* is present at least once, are, so far, the only two data supporting, for the *blood*-induced mutations, a model similar to the one for the suppression of the *gypsy*-induced mutations.

Independently of whether these explanations are correct, we would like to stress two points: (1) the hypothesis presented can be tested experimentally in several ways and can represent a frame of reference for future experiments; (2) some, or most, of the features attributed to the *abo* mutation may not be characteristic of the *abo* gene *per se* but of the specific allele that has been studied which may be due to the presence of *blood* transposon in region 32E.

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