RIBOSOMAL GENE MAGNIFICATION IN DROSOPHILA: A CHROMOSOMAL CHANGE

A. GAYLER HARFORD¹

Department of Biology, Yale University, New Haven, Connecticut 06520

Manuscript received November 30, 1973

Revised copy received June 18, 1974

ABSTRACT

The sex-linked mutant "bobbed" can undergo a rapid phenotypic reversion during a prescribed series of outcrosses. The experiments reported here distinguish between two general genetic models. The first is that the phenotypic change results from the changing genetic background brought about by the outcrosses. The second is that the phenotypic change results from an alteration of the *X* chromosome in the germ line. The data support the second model. It is shown that both the bobbed and reverted phenotypes segregate from the same female. In addition, the reverted phenotype maps in or near the proximal heterochromatin of the *X* chromosome, which is the standard map position of both bobbed and the nucleolus organizer.

THE ribosomal genes have the unusual characteristic of being able at times to replicate independently of the rest of the genome. These genes replicate disproportionately during oogenesis in a number of organisms (for review see BROWN and DAWID 1969). Independent replication can also occur in somatic tissues of *Drosophila* (HENNIG and MEER 1971; TARTOF 1971; SIBATANI 1972; SPEAR and GALL 1973). This paper investigates the genetic basis of a phenomenon which may be involved in the regulation of the degree of reiteration of these genes.

In *Drosophila melanogaster* there exists a class of alleles called bobbed (*bb*), characterized by short bristles, abdominal etching, delayed development, and reduced viability and fertility (see LINDSLEY and GRELL 1967). *bb* is located at or near the nucleolus organizer in the proximal heterochromatin of the *X* chromosome and on the *Y* chromosome (COOPER 1959). RITOSSA, ATWOOD and SPIEGELMAN (1966) proposed that the bobbed phenotype results from an insufficient number of ribosomal genes.

RITOSSA (1968) reported that the bobbed phenotype can revert over the course of several generations during a particular series of outcrosses (Figure 1). Sons of bobbed males appear less bobbed than their fathers despite the fact that both carry the same sex chromosomes. From molecular hybridization experiments, RITOSSA (1968) proposed that the changed phenotype results from an increased reiteration of ribosomal genes. The phenomenon was termed "magnification".

¹ Present address: Division of Cell and Molecular Biology, State University of New York at Buffalo, Buffalo, New York 14214.

Although the hybridization data are difficult to interpret (see DISCUSSION), the hypothesis remains attractive.

In any case, it has become increasingly clear that the degree of reiteration of the ribosomal genes in the germ line cannot be directly inferred from these hybridization measurements because the DNA was isolated from whole flies. The difficulty in interpretation arises because of the possibility of independent replication of these genes in somatic tissues. That differential replication of ribosomal genes might occur in different tissues of *Drosophila* was first suggested in the work of HENNIG and MEER (1971) using *Drosophila hydei*. They found different reiterations in embryos, polytene salivary glands of larvae, and adults. The value for whole third instar larvae also differs from that of adults (SIBATANI 1972). Differential replication can result in compensation. TARTOF (1971) found that the reiteration of ribosomal genes in an *XO* fly was about 80% that of an *XX* fly instead of the 50% expected from the fact that the nucleolus organizers are confined to the sex chromosomes in this organism. Recent work indicates that compensatory synthesis of ribosomal genes can be tissue-specific (SPEAR and GALL 1973). If brains and imaginal disks of larvae are examined instead of whole flies, the reiteration of the *XO* is found to be 50% of that of the *XX*. On the other hand, the reiteration of these genes is the same in the salivary glands of *XO* and *XX* larvae. Thus DNA isolated from whole flies does not represent an invariant genome which is present in every cell of the organism, but rather the end product of differential replication of different DNA sequences in a varied collection of somatic tissues. Consequently, the precise genetic basis of the phenotypic reversion described by RITOSA remains in doubt.

My experiments distinguish between two general models which have different implications for our notions of how ribosomal gene number might be controlled. The first model is that the phenotypic change results from an alteration of the *X* chromosome in the germ line. One possibility is a change in the number of ribosomal genes carried by the *X* chromosome. Such a change in gene number might or might not be fully reflected in the average ribosomal gene reiteration of DNA from whole flies because such changes might be compensated for on the somatic level (TARTOF 1971; SPEAR and GALL 1973).

The second model is that the different phenotypes result from the interaction of the bobbed locus with different genetic backgrounds. The weakly bobbed and wild-type phenotypes seen after repeated outcrossing to another stock are understandable if the latter stock contains autosomal modifiers of bobbed. This latter stock originally carried bobbed mutants of its own (see MATERIALS AND METHODS), and so it may have been under strong selective pressure to accumulate such modifiers. This model makes no specific prediction about ribosomal gene numbers. It is not known to what extent genetic background might affect the measurements made on DNA from whole flies either by affecting differential replication of these genes in somatic tissues or by altering the proportions of different types of cells. On obvious possibility is that the modifiers might affect the phenotype through their effect on somatic gene reiteration. However, the important feature of the model that distinguishes it from the first model is that there is

no change in the amount or content of the information carried in individual chromosomes in the germ line. Instead, the phenotypic changes and any changes in gene reiteration at the somatic level which might accompany them result from new combinations of chromosomes.

To distinguish between these two models females were constructed with two distinctively marked *X* chromosomes which were identical in the bobbed region except that one chromosome had experienced the series of "magnification" crosses and the other had not. These females were mated in such a way that the inheritance of the bobbed and reverted phenotypes could be studied in the male progeny. The chromosomal change hypothesis predicts that the bobbed and reverted phenotypes should segregate and show linkage with appropriate markers on the *X* chromosome. The genetic background hypothesis, on the other hand, predicts that no such linkage should be observed since autosomal modifiers should assort independently of markers on the *X* chromosome. As will be seen, the data support the chromosomal change hypothesis. The chromosomal change is found to map in or near the proximal heterochromatin of the *X* chromosome, which is the map position of both *bb* and the nucleolus organizer.

MATERIALS AND METHODS

Genetic symbols are those used by LINDSLEY and GRELL (1967). Flies were grown on standard cornmeal molasses agar DOANE 1967) supplemented with yeast (Fleischmann's mfd. by Standard Brands Inc., New York, N.Y. 10022).

The following stocks of *Drosophila melanogaster* were used:

1. *w sn bb / C(1)RM, γ v f / Y^{-bb}*, obtained from Bowling Green State University, Department of Biology, Bowling Green, Ohio (stock no. f59). Males in this stock do not appear bobbed.
2. *C(1)RM, γ v bb / Y^{SX} · Y^L In(1)EN, Y^{SB} f v γ · Y^L γ + / 0*
3. *Basc: In(1) sc^{SIL} sc^S R + S, sc^{S1} sc^S w^a B*
from the Yale University collection (stock no. 35).
4. *Basc: In(1) sc^{SIL} sc^S R + S, sc^{S1} sc^S w^a B;*
SM1: In(2LR) SMI, al² Cy cn² sp² / dp b Pm ds^{33K};
TM2: In(3LR) Ubx¹³⁰, Ubx¹³⁰ e^S / C Sb
from the Yale University collection (stock no. 107).
5. *sn^S v B* from the Yale University collection (stock no. 38).
6. *g² ty / C(1)DX, γ f / Y* obtained from California Institute of Technology (#54). This stock was found to contain a strong bobbed allele, as reported by RIRROSSA (1968). Stock was restarted from a single male. A second stock was started from a *g² ty* sibling of the *sn^S v* male which was used to start stock 7. This latter *g² ty* stock was the one used in the cross shown in Tables 4 and 6.
7. *sn^S v bb / C(1)DX, γ f / Y*. This stock was prepared in the following way: *g² ty bb / Y* males were mated to *sn^S v B bb + / sn^S v B bb +* females (stock no. 38, Yale University, New Haven, Connecticut) to produce *g² ty bb / sn^S v B bb +* females. These females were then mated to *g² ty bb / Y* males. Individual male progeny that were either *sn^S v B +* or *g² ty B +* were selected and a stock started from each by mating to *C(1)DX, γ f / Y*. In addition, each male was tested for bobbed by mating with *C(1)RM, γ v bb / 0*.

RESULTS

Construction of the Female Parents

To study the inheritance of the bobbed and reverted phenotypes, an *X* chromosome which has undergone the series of "magnification" crosses is placed in a female with an *X* chromosome that has not. In the first experiment such females of genetic constitution $g^2 ty bb$ (magnified) / $++ bb$ (bobbed) were produced in the following way: females of genetic constitution *Basc/Basc*; *Cy/Pm*; *Ubx/Sb* (stock no. 4 in MATERIALS AND METHODS) were mated to males $g^2 ty bb/Y$ to produce female progeny $g^2 ty bb/Basc$. Because of the presence of autosomal inversions, a high rate of crossing over between $g^2 ty bb$ and the doubly inverted *Basc* occurs in these females (SCHULTZ and REDFIELD 1951). The recombination and segregation of bobbed which occurs in such females is shown in Table 1. It can be seen that w^a is closely linked to bb^+ , as would be expected.

Females $g^2 ty bb/Basc$ (with various combinations of autosomes) were mated to males $g^2 ty bb/Y^{-bb}$ (weakly bobbed "magnified" flies, line 4 of Figure 1). By selecting female progeny with wild-type eyes from this cross, one gets with high probability $g^2 ty bb$ (magnified) / $++ bb$ (bobbed). A few $B^+ w^a bb^+$ chromosomes will be picked up, but these can be easily spotted by the appearance of w^a in the subsequent male progeny.

In the second experiment females of genetic constitution $g^2 ty bb$ (magnified) / $sn^3 v bb$ (bobbed) and $sn^3 v bb$ (magnified) / $g^2 ty bb$ (bobbed) were constructed in the following way: Males $sn^3 v bb/Y$ and $g^2 ty bb/Y$ were mated with females *Basc/Basc* (no autosomal inversions in this stock) to produce female progeny *Basc/sn³ v bb* and *Basc/g² ty bb*, respectively. These females were then mated to

TABLE 1

Results of cross

Basc: In (1) sc^{SIL} sc^{SR} + S, sc^{S1} sc^8 w^aB		$\frac{SM1}{+}$; $\frac{TM2}{+}$ $\times$ $\frac{w sn}{Y^{-bb}}$ $\frac{+}{+}$; $\frac{+}{+}$	
$g^2 ty bb$			
X-chromosome genotype (phenotype)		Nonbobbed flies	Bobbed flies
Males:	w^aB (apricot-bar)	169	0
	w^a (apricot)	6	0
	$w^a g^2B$ (white-bar)	31	0
	$w^a g^2$ (white)	27	1
	g^2 (garnet)	0	34
	+	0	15
	<i>B</i> (bar)	0	13
	$w sn$ (white singed)*	64	0
Females:	w^aB (apricot-bar)	199	0
	w^a (apricot)	40	0
	+	209	0
	<i>B</i> (bar)	34	0

* *Patroclinus XO* males.

FIGURE 1.—Crosses leading to "magnification". Shown are percent of DNA which is complementary to ribosomal RNA. Value in parenthesis is percent which is attributed to *g² ty bb* chromosome. Values taken from RIROSSA *et al.* (1971).

males *g² ty bb/Y^{-bb}* and *sn^s v bb/Y^{-bb}* (weak bobbed "magnified" flies, line 4, Figure 1), respectively, to produce female progeny *g² ty bb* (magnified)/*sn^s v bb* (bobbed) and *sn^s v bb* (magnified)/*g² ty bb* (bobbed).

Inheritance of the Magnified Condition

The females with bobbed and "magnified" chromosomes were then mated to males *Y^SX · Y^LIn (1) En, Y^SB f v/O, Basc/Y^{-bb}*, or *w sn/Y^{-bb}* so that the inheritance of the bobbed and reverted phenotypes could be studied in the male progeny. The male progeny could be easily divided into two classes on the basis of abdominal etching: (1) "magnified" flies: no etching or slight etching, and (2) bobbed flies: severe etching. The results are shown in Tables 2–6.

Table 2 shows the male progeny of females *g² ty bb* (magnified)/++ *bb* (bobbed). It can be seen that the "magnified" and bobbed phenotypes are linked to the *g* alleles on their respective chromosomes and that the frequencies of recombination agree quite well with the standard map distance between *g* and *bb* (22 units). These results support the notion that the "magnified" phenotype

TABLE 2

Male progeny from female parent g^2 ty bb (magnified)

+ + bb (bobbed)

	g	g^+	Recombination frequency
Magnified	417	127	.23 $\pm$.02
Bobbed	31	114	.21 $\pm$.03

results from a chromosomal change rather than from autosomal modifiers since such modifiers would be expected to assort independently of the two X chromosomes and thus no linkage with the g locus should have been observed. The agreement between the recombination frequencies found for both "magnified" and bobbed flies is strong evidence that the result is not an artifact resulting from differential viability associated with other markers on the X chromosome.

The chromosomal change model was further confirmed in the second experiment. Here the female parents were g^2 ty bb (magnified)/ sn^3 v bb (bobbed) and sn^3 v bb (magnified)/ g^2 ty bb (bobbed). The results are shown in Tables 3-6. The tables list the complementary crossover classes in pairs and the frequency predicted for each class under the two models. The genetic background theory predicts that complementary crossover classes should occur with the same frequency. By contrast, the chromosomal change theory predicts that the frequencies of the complementary classes should be grossly unequal. The direction of the inequality depends upon which of the two X chromosomes has experienced the series of "magnification" crosses. Thus the reciprocal placement of markers

TABLE 3

Magnified male progeny from female parent g^2 ty bb (magnified) sn v bb (bobbed)

Genotype	No. males	Frequency	Expected frequencies	
			Linkage	No linkage
g	627	.54	.55	.39
sn v	242	.21	.20	.39
wild	12	.01	<.02	.05
sn v g	106	.09	.10	.05
v	30	.03	.02	.06
sn g	138	.12	.12	.06
v g	9	.008	<.01	<.01
sn	0	.00	<.002	<.01
Total	1164			

TABLE 4

Magnified male progeny from female parent

Genotype	No. males	Frequency	Expected frequencies	
			Linkage	No linkage
<i>g</i>	76	.19	.20	.39
<i>sn v</i>	225	.56	.55	.39
wild	34	.09	.10	.05
<i>sn v g</i>	6	.02	<.02	.05
<i>v</i>	53	.13	.12	.06
<i>sn g</i>	7	.02	.02	.06
<i>v g</i>	0	.00	<.002	<.01
<i>sn</i>	0	.00	<.01	<.01
Total	401			

in the two female parents provides an internal control for possible effects of *X* chromosome markers on viability or expression of the bobbed phenotype.

Tables 3-4 show that for flies with the "magnified" phenotype the frequencies of the various classes agree quite well with those predicted by the chromosomal change theory and quite poorly with the genetic background theory. The overall recombination frequencies between the magnified phenotype and *g* are $.24 \pm .01$ and $.22 \pm .02$ for Tables 3 and 4 respectively.

Possible errors in scoring the magnified and bobbed phenotypes do not affect the major conclusion, for even if both magnified and bobbed flies are pooled and only survival taken as an index of magnification, linkage with *g* is still observed (recombination frequencies are .30 and .34).

Tables 5 and 6 show the frequencies of the various classes of bobbed flies. Here too the results agree more closely with the chromosomal change theory than with the genetic background theory. However, the overall recombination frequencies with *g* are somewhat higher than expected ($.36 \pm .04$ and $.32 \pm .04$ for Tables 5 and 6 respectively). It is unlikely that these high frequencies are due to differential viability of different markers since the two reciprocal crosses give the same results. The possibility that some of the grandfathers (line 4, Figure 1) carried bobbed loci which had failed to undergo magnification seems unlikely also since all of these males showed a magnified phenotype. A possible explanation is that the magnified state is unstable and a certain fraction of the chromosomes loses its magnified state in the female parent. Instability of the magnified state has been reported previously (RROSSA 1968). It can be calculated that the observed amount of recombination with *g* would result if one-third of the magnified chromosomes had reverted to the bobbed state. However, it is conceivable that the apparent increase in recombination is an artifact resulting from the par-

TABLE 5

Bobbed male progeny from female parent

Genotype	No. males	Frequency	Expected frequencies	
			Linkage	No linkage
<i>g</i>	59	.33	.20	.39
<i>sn v</i>	72	.40	.55	.39
wild	17	.10	.10	.05
<i>sn v g</i>	2	.01	<.02	.05
<i>v</i>	24	.13	.12	.06
<i>sn g</i>	3	.017	.02	.06
<i>v g</i>	0	.00	<.002	<.01
<i>sn</i>	1	.006	<.01	<.01
Total	178			

TABLE 6

Bobbed male progeny from female parent

Genotype	No. males	Frequency	Expected frequencies	
			Linkage	No linkage
<i>g</i>	72	.53	.55	.39
<i>sn v</i>	32	.24	.20	.39
wild	4	.029	<.02	.05
<i>sn v g</i>	9	.066	.10	.05
<i>v</i>	8	.059	.02	.06
<i>sn g</i>	11	.081	.12	.06
<i>v g</i>	0	.00	<.01	<.01
<i>sn</i>	0	.00	<.002	<.01
Total	136			

ticular criterion selected for scoring the phenotypes and may not reflect any instability at the chromosomal level. Experiments are in progress to clarify this point.

DISCUSSION

The ability of an organism to correct undesirable features of its genome is of considerable interest. For this reason, I felt it to be especially important that the genetic basis of the magnification phenomenon be rigorously established and that

all possible sources of artifact be eliminated. The present work demonstrates the existence of a chromosomal alteration in a direct and simple way. By putting the two *X* chromosomes to be compared in the same female, not only is genetic background controlled but so, also, are other possible variables such as culture conditions and parental effects.

At present the most attractive hypothesis is that the genetic alteration involves a change in the reiteration of the ribosomal genes in the inherited genome, as originally proposed by RITOSSA (1968). However, this remains to be rigorously established. Alternatively one could imagine that the genetic change influences, either directly or indirectly, the replication of these genes in somatic tissues.

In any case, much work is needed to clarify the relationship between the reiteration in the inherited genome and that in the somatic tissues, as well as the relationship between gene reiteration and phenotype. It is beyond the scope of this paper to discuss in detail the relative merits of specific models. However, some difficulties in the interpretation of current data should be noted. One must be cautious in interpreting the measurements made on whole flies involved in the "magnification" crosses (RITOSSA *et al.* 1971). (See Figure 1 for a summary of these data.) The reiteration associated with the original bobbed locus was determined by subtracting from the total ribosomal gene reiteration that presumed to be associated with the *Y* chromosome in this fly. This latter value is large compared with that attributed to the *X*. Thus a small error in determination of the *Y* value or a small departure from strict additivity of reiteration values would affect the value attributed to the *X* chromosome considerably. When bobbed and reverted flies having the same sex chromosomes are compared, the reiteration differences are rather small. In addition, some puzzling features of the data should be noted. Flies *g² ty bb/0* (line 2 in Figure 1) have a greater ribosomal gene reiteration than previously thought necessary for a normal phenotype, and yet they appear strongly bobbed. Flies of the type *g² ty bb/Y^{-bb}* show essentially the same reiterations as their *g² ty bb/0* counterparts, and yet the *Y^{-bb}* chromosome has been postulated to carry inoperative ribosomal genes (RITOSSA 1968). Furthermore, when a *bb* locus is maintained for several generations in a female line opposite an *X* chromosome completely deficient for *bb*, ribosomal gene number increases to wild-type level without any concomitant change in the bobbed phenotype (TARTOF 1973).

The resolution of these problems may have implications for our understanding of not only the magnification phenomenon and perhaps other examples of directed mutation, but also of chromosome structure, evolution of repeated DNA sequences, and the molecular prerequisites for gene activity.

This investigation was carried out during the tenure of an NIH postdoctoral fellowship (No. GM 52418) in the laboratory of DR. JOSEPH G. GALL, Yale University. Research was supported by an NIH Research Grant No. GM 12427 to DR. JOSEPH G. GALL.

I would like to thank DR. GALL, DR. BRIAN SPEAR and DR. WALTER GEHRING for their valuable discussions and insights. I would like to thank DR. GALL in particular for his support and advice. In addition, I would like to thank DR. GALL, DR. SPEAR and DR. WINIFRED DOANE for reading the manuscript.

This work was presented at the Thirteenth Annual Meeting of the American Society for Cell Biology, November 1973, and published in abstract form (J. Cell. Biol. **59**: 133A (1973)).

LITERATURE CITED

- BROWN, D. and I. DAWID, 1969. Developmental genetics. Ann. Rev. Genet. **3**: 127-154.
- COOPER, K. H., 1959. Cytogenetic analysis of major heterochromatic elements (especially Xh and Y) in *Drosophila melanogaster*, and the theory of "heterochromatin". Chromosoma **10**: 535-588.
- DOANE, W. W., 1967 pp. 219-44. In: *Methods in Developmental Biology*. Edited by F. H. WILT and N. K. WESSELS. Thomas Y. Crowell, N.Y.
- HENNIG, W. and B. MEER, 1971. Reduced polyteny of ribosomal RNA cistrons in giant chromosomes of *Drosophila hydei*. Nature New Biol. **233**: 70-72.
- LINDSLEY, D. L. and E. H. GRELL, 1967. *Genetic Variations of Drosophila Melanogaster*. Carnegie Institution of Washington Publication No. **627**.
- RITOSSA, F. M., 1968. Unstable redundancy of genes for ribosomal RNA. Proc. Natl. Acad. Sci. U.S. **60**: 509-516.
- RITOSSA, F. M., K. C. ATWOOD and S. SPIEGELMAN, 1966. A molecular explanation of the bobbed mutants of *Drosophila* as partial deficiencies of "ribosomal" DNA. Genetics **54**: 819-834.
- RITOSSA, F., C. MALVA, E. BONCINELLI, F. GRAZIANI and L. POLITO, 1971. The first steps of magnification of DNA complementary to ribosomal RNA in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **68**: 1580-1584.
- SCHULTZ, J. and H. REDFIELD, 1951. Interchromosomal effects on crossing over in *Drosophila*. Cold Spring Harbor Symp. Quant. Biol. **16**: 175-195.
- SIBATANI, A., 1972. Difference in proportion of DNA specific to ribosomal RNA between adults and larvae of *Drosophila melanogaster*. Molec. Gen. Genetics **114**: 177-180.
- SPEAR, B. and J. G. GALL, 1973. Independent control of ribosomal gene replication in polytene chromosomes of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **70**: 1359-1363.
- TARTOF, K. D., 1971. Increasing the multiplicity of ribosomal RNA genes in *Drosophila melanogaster*. Science **171**: 294-297. —, 1973. Regulation of ribosomal RNA gene multiplicity in *Drosophila melanogaster*. Genetics **73**: 57-71.

Corresponding editor: B.H. Judd