AUTOSOMAL MODIFIERS OF THE BOBBED PHENOTYPE ARE A MAJOR COMPONENT OF THE rDNA MAGNIFICATION PARADOX IN DROSOPHILA MELANOGASTER

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ABSTRACT

rDNA magnification in Drosophila melanogaster is defined experimentally as the ability of bb/Ybb- males to produce exceptional progeny that are wild type with respect to rDNA associated phenotypes. Here, we show that some of these bobbed-plus progeny result not from genetic reversion at the bb locus but rather from variants at two or more autosomal loci that ameliorate the bobbed phenotype of rDNA deficient males in Drosophila. In doing so we resolve several aspects of a long-standing paradox concerning the phenomenon of rDNA magnification. This problem arose from the use of two genetic assays, which were presumed to be identical, but paradoxically, produced conflicting data on both the kinetics of reversion and the stability of magnified bb+ chromosomes. We resolve this problem by demonstrating that in one assay bobbed-plus progeny arise primarily by genetic reversion at the bobbed locus, whereas in the other assay bobbed-plus progeny arise both by reversion and by an epistatic effect of autosomal modifiers on the bobbed phenotype. We further show that such modifiers can facilitate the appearance of phenotypically bobbed-plus progeny even under conditions where genetic reversion is blocked by magnification defective mutants. Finally, we present a speculative model relating the action of these modifiers to the large increases in rDNA content observed in males undergoing magnification.

IN genetically normal Drosophila melanogaster males there are two clusters of tandemly repeated rRNA genes (rDNA), each containing approximately 250 genes. One of these arrays is located in the proximal heterochromatin of the X chromosome and the other on the short arm of the Y chromosome. Partial deficiencies at either cluster are known as bobbed (bb) mutants. These mutants range in severity from very weak alleles to lethal mutations (bb') according to the extent of the deletion. Complete (bb^0) and near-complete (bb^-) deficiencies are also available for both the X and Y chromosomal bb loci. Individuals of the genotype bb/bb^0 or bb/bb^- display a strong bobbed phenotype that includes extremely short and thin bristles, etching of the abdominal...
The \(se^4se^8\) assay for magnification

(TARTOF 1973)

\[bb/Ybb^- \times C(1)RM/Ybb^-\]

\(G_0\)

\[bb/Ybb^- \times se^4se^8/dl-49\]

\(G_1\)

\(X/scar\) progeny for \(bb^+\) vs. \(bb\)

Properties
1. Some fraction (15%) of \(X\) chromosomes show reversion to \(bb^+\).
2. Reversion is frequently complete within one generation.
3. Reversion observed at \(G_1\) is usually stable and heritable (TARTOF 1973; HAWLEY and TARTOF 1985).
4. Revertants are highly clustered among the progeny of individual males (TARTOF 1973).
5. Does not require \(mei-9^+\) (HAWLEY and TARTOF 1983).
7. Does not occur in ring chromosomes (TARTOF 1974).

The \(Ybb^-\) assay for magnification

(RITOSSA 1968a)

\[bb/Ybb^- \times C(1)RM/Ybb^-\]

\(G_0\)

\[bb/Ybb^- \times C(1)RM/Ybb^-\] (from stock)

\(G_1\)

\[bb/Ybb^- \times C(1)RM/Ybb^-\] (from stock)

\(G_0\)

\[bb/Ybb^- \times C(1)RM/Ybb^-\] (from stock)

Score \(X/Ybb^-\) males for \(bb^+\) vs. \(bb\)

Properties
1. Uniform decrease in severity of \(bb\) phenotype among the progeny of magnifying males.
2. \(bb\) severity usually decreases steadily with increasing generations. However, cases of complete reversion within a single generation have been observed (BONCINELLI et al. 1972).
3. bobbed-plus progeny found in \(G_1\) frequently fail to carry stable and heritable \(bb^+\) alleles.
4. No evidence of clustering.
5. Requires \(mei-9^+\) (POLITO et al. 1982; but see the contradictory results presented here).
6. Not inhibited by \(mei-41, mus-108\) (this manuscript).

**Figure 1.**—A comparison of the \(se^4se^8\) and \(Ybb^-\) assays for rDNA magnification.

Tergites, and lengthened developmental time. Although rDNA redundancy is generally quite stable (TARTOF 1974), alterations in \(X\) chromosomal rDNA occur at high frequencies in the germlines of males carrying a bobbed-deficient \(Y\) chromosome (\(Ybb\) or \(Ybb^-\)). These changes in rDNA redundancy may be observed as either reversions of \(bb\) to \(bb^+\) (magnification, RITOSSA 1968a and TARTOF 1973) or as mutations from \(bb^+\) to \(bb\) or from \(bb\) to \(bb^-\) (reduction, TARTOF 1974).

Magnification has been analyzed by two different genetic assays which are diagramed and described in Figure 1. In the first assay, \(bb/Ybb^-\) males are crossed to \(In(1)se^{4fs8R}/In(1)dl-49\) females. The \(In(1)se^{4fs8R}\) chromosome carries a complete deficiency for \(bb\) (\(bb^0\)), and therefore, magnification may be observed by examining the phenotype of the \(X/In(1)se^{4fs8R}\) daughters. (\(In(1)dl-49\) is a \(bb^+\) balancer chromosome that need not be considered further.) This assay was exploited both by RITOSSA (1968a) and by TARTOF (1974) and will henceforth be referred to as the \(se^4se^8\) assay. In the second assay, \(bb/Ybb^-\) males are backcrossed to \(C(1)RM/Ybb^-\) females from the maternal stock, and the \(X/Ybb^-\) sons are scored with respect to \(bb\). This assay was introduced by RITOSSA (1968a) and will be referred to as the \(Ybb^-\) assay. Both assays are characterized by an increase in rDNA redundancy in the \(bb^+\) progeny. Although both assays measure magnification by placing an \(X\) chromosome de-
AUTOSOMAL MODIFIERS OF BOBBED

rived from the bb/Ybb− male over an rDNA-deficient (bb0 or bb−) homologue, they have produced conflicting data regarding the genetic basis of the magnification process (see Figure 1). Perhaps the most important differences are the frequency of magnification, the heritability of magnification, and the effects of some magnification-defective mutants. It occurred to us that these differences might result from a variety of causes; namely, the use of different alleles of bb by various investigators; the fact that in one assay females are scored, whereas in the other assay males are scored; the presence of different strain backgrounds; or different criteria used to score bb. To examine this problem we measured magnification of a single well-characterized bb allele (bb2, TARTOF 1973) using both assays. To eliminate any subjective bias in scoring the bobbed phenotype, we used as our metric the length of the posterior scutellar bristles. For both assays, our data confirm the observations made by previous investigators. We find that in the sc4sc8 assay the majority of the X/sc4sc8 progeny are still strongly bobbed, whereas the remainder appear as bobbed-plus progeny that carry a stable and heritable X chromosomal bb+ locus. On the other hand, in the Ybb− assay the vast majority of X/Ybb− progeny are less severely bobbed than their bb/Ybb− fathers. However, on retesting, the majority of these males are shown to still carry bb mutations.

To explain these data we hypothesized that a substantial fraction of the bb+ male progeny observed in the Ybb− assay arise by a process or mechanism not revealed by the sc4sc8 assay; namely, the accumulation of autosomal modifiers of the bb phenotype present in the C(1)RM/Ybb− stock. This hypothesis was verified by demonstrating that at least two stocks of X/Ybb− males and C(1)RM/Ybb− females carry autosomal modifiers that can strongly ameliorate the bobbed phenotype of bb/Ybb− males. Indeed, these modifiers allow phenotypic reversion even under conditions in which bb to bb+ genetic reversion is blocked by magnification-defective mutations.

MATERIALS AND METHODS

Stocks: The flies were raised at 24.5° on standard medium (TARTOF 1973). Complete descriptions of most of the mutants used in this study, except mei-41, mus-108 and mei-9, may be found in LINDSLEY and GRELL (1968). The pertinent chromosomes used here are as follows: In(1)sc4sc8bb, sc4sc8 cv B (sc4sc8) is an inverted-X chromosome completely deficient for rDNA; bb2 arose spontaneously in K. D. TARTOF's laboratory and is an X chromosomal bb mutant deficient for 47% of its rDNA (TARTOF 1973); Ybb− is a Y chromosome deficient for at least 80% of its rDNA (TARTOF 1973); B′Ybb−, which carries the Ybb− deletion on Y and B′ on Y′, was derived by D. KOMMA as a consequence of exchange between Ybb− and a B′-bearing Y chromosome; C(1)DX,y f is an attached-X chromosome completely deficient for rDNA; C(1)RM,y w is an attached-X chromosome that carries bb+; C(1)RM,Y is an attached-X chromosome that carries bb+ and w+Y; In(1)dl-49,Y Hu m2g4, (dl-49), is a bb+ inversion-bearing X chromosome; B′Y and Y′Y are bb+ Y chromosomes, the long arms of which are marked with B′ or Y′; the meiotic mutants mei-41 and mei-9 were obtained from L. SANDLER; the mutagen-sensitive mutation mus-108 was obtained from J. BOYD.

Bristle measurements: The posterior-scutellar bristle lengths of 15- to 16-day-old progeny were measured using a Bausch & Lomb Ocular Micrometer mounted in a standard stereomicroscope. Bristle lengths were measured at 7× magnification, such that an arbitrary unit on the micrometer corresponds to 35.8 microns in length. Each
bristle was measured twice, and the four values obtained were averaged to give the measurement for each individual scored. Only measured individuals were used to sire successive generations.

**Assays for magnification:** The basic tests for magnification are described in Figure 1. The tester female in the sc^4sc^8 assay is sc^4sc^8/dl-49. These females are kept in a stock of dl-49/B'Ybb^- males crossed to sc^4sc^8/dl-49 females to prevent the accumulation of Y chromosomes in the tester female. Since sc^4sc^8 carries B (a dominant eye-shape marker), X/sc^4sc^8 daughters can easily be identified and scored with respect to X/Ybb^- males crossed to C(I)RM,w/BYbb^- females.

The bb^2/BY males were from a stock of bb^2/BY males crossed to C(1)DX,y f/BY females. This stock was recently derived from a single bb^2/BY male from a stock of bb^2/BY males crossed to bb^2/bb^2 females. The bb^2 stock was obtained from K. D. Tartof.

**Test crosses of magnified progeny:** The following schemes were devised to allow phenotypically wild-type progeny from each assay to be testcrossed in a similar fashion, such that in each case the X chromosome has been removed from magnifying conditions for at least one generation and the measured descendants are of the same genotype in each assay. Phenotypically wild-type (X/B'Ybb^-) males from the Ybb^- assay were first crossed to C(I)DX,y f/B'Ybb^- females. These females were obtained from a stock of sc^4sc^8/B'Y males crossed to C(I)DXg f/BsY females. The X/EY sons were then crossed to sc^4sc^8/dE-49 females, and the X/sc^4sc^8 daughters were used for bristle measurements.

For the sc^4sc^8 assay, phenotypically wild-type daughters were crossed to sc^4sc^8/EY males. These males were derived from the same stock of sc^4sc^8/B'Y males crossed to C(I)DX,y f/B'Y females that had been used in the testcrosses for the Ybb^- assay. The X/sc^4sc^8 daughters of this mating were then used for bristle measurements.

**RESULTS**

The basic crosses reported here are diagramed in Figure 1. Males of the genotype bb^2/BY were mated to C(1)RM,w/BYbb^- females, and the sons were then mated on day one to either sc^4sc^8/dl-49 or C(1)RM,w/BYbb^- females. Progeny were collected until day 16; then the bristle lengths were determined as described in MATERIALS AND METHODS. To obtain data for the Go bobbed animals, bb^2/BY males were mated directly to the two classes of tester females. The bobbed-plus control data for the sc^4sc^8 assay were obtained from a cross of Ore-R bb^+/Ybb^+ males to sc^4sc^8/dl-49 females to produce Xbb^+/sc^4sc^8 females. The bobbed-plus control data for the Ybb^- assay were obtained simply from bb^2/BY males. Further generations of the Ybb^- assay resulted from mating all measured males to C(1)RM,w/BYbb^- virgin females from stock.

**The two assays differ in terms of their kinetics:** We first consider magnification in bb^2 males as measured by the sc^4sc^8 assay (Figure 2, panel A). The data for males carrying mei-9, mei-41 or mus-108 will be considered later. The upper figure in panel A displays the bristle length histogram for bb^2/sc^4sc^8 progeny derived from nonmagnifying bb^2/BY males (G0), as well as the bristle length histogram from control bb^+/sc^4sc^8 females. The lower figure in panel A displays the distribution of bristle lengths in X/sc^4sc^8 female progeny of magnifying bb^2/B'Ybb^- (G0) males. It may be seen that the majority of the progeny obtained from bb^2/B'Ybb^- males were phenotypically similar to those obtained from nonmagnifying (bb^2/BY) males. Indeed, the modes of the two distributions are identical. However, bb^2/B'Ybb^- males produced a significant
AUTOSOMAL MODIFIERS OF BOBBED

fraction (10%) of individuals with wild-type bristle lengths. These individuals were characteristically devoid of the other stigmata of the bobbed phenotype and were scored as bobbed-plus. Thus, as measured by the \( sc^4sc^8 \) assay, magnification is a discrete process resulting in phenotypic reversion of a small fraction of the \( X/sc^4sc^8 \) progeny. Brothers of the \( bb^2/B^Yybb^- \) males used in the \( sc^4sc^8 \) assay were also tested in a \( Ybb^- \) assay, and the results are presented in Figure 3. The top figure (panel A) displays the bristle length distributions for \( G_0 bb^2/B^Yybb^- \) males and for \( bb^2/B^Y \) males that may be used as a \( bb^+ \) control. Since \( G_0 bb^2/B^Yybb^- \) males were derived from nonmagnifying \( bb^2/B^Y \) fathers, they serve as our standard for the \( bb \) phenotype. The lower figures in panel A (marked \( G_1, G_2 \) and \( G_3 \)) display the bristle length histograms for successive generations of magnification. Unlike the \( sc^4sc^8 \) assay, in the \( Ybb^- \) assay the majority of scored \( G_1 \) progeny were much less severely bobbed in phenotype than were their \( G_0 \) fathers. Moreover, the extent of magnification in the \( Ybb^- \) assay increased with succeeding generations until, by \( G_3 \), the bristle length...
AVERAGE BRISTLE LENGTH

Figure 3.—Magnification in the Ybb⁻ assay. Bristle length histograms of X/B⁺Ybb⁻ progeny of males carrying the indicated X chromosome crossed to C(1)RM,w/B⁺Ybb⁻ females. G₀ males result from crossing bᵇ²/B⁺ (nonmagnifying) males to C(1)RM,w/B⁺Ybb⁻ females. G₁ males are derived by backcrossing measured bᵇ²/B⁺Ybb⁻ (magnifying) G₀ males to C(1)RM,w/B⁺Ybb⁻ females. Successive generations are derived by repetitive backcrossing of bᵇ²/B⁺Ybb⁻ measured males to C(1)RM,w/B⁺Ybb⁻ virgin females from stock. In each case the control, depicted by the crosshatched distribution in the G₀ panel, represents the distribution for X/F⁺Y males.

distribution has little overlap with that observed at G₀. Changes in another characteristic of the bobbed phenotype, abdominal etching, paralleled the increases in bristle length. The percentage of bᵇ²/B⁺Ybb⁻ males showing etching decreased, from 100% at G₀, to 69% at G₁, to 10% at G₂, and to 3% at G₃. Thus, magnification in the Ybb⁻ assay appears to be a general phenomenon that affects the majority of the offspring rather than the less frequent event observed in the sc⁴sc⁸ assay.

The two assays differ in terms of the heritability of the bobbed-plus phenotype: We have also compared the sc⁴sc⁸ and Ybb⁻ assays with respect to the heritability of the bobbed-plus phenotype. In the experiments involving the bᵇ² chromosome, 17 phenotypically bobbed-plus X/sc⁴sc⁸ females and 24 phenotypically bobbed-plus X/B⁺Ybb⁻ males were retested as described in MATERIALS AND METHODS. Following one generation under nonmagnifying conditions, the bristle lengths of at least five X/sc⁴sc⁸ descendants from each individual were determined. In all cases, each chromosome gave a uniform bristle length upon retesting, and in no case were both bᵇ and bᵇ⁺ progeny derived from a single individual. A histogram of the average bristle lengths for the descendants of each retested chromosome is presented in Figure 4. We first consider the testcross data for the sc⁴sc⁸ assay. It may be seen that 88% (15 of 17) of the bobbed-plus X/sc⁴sc⁸ females produced X/sc⁴sc⁸ descendants with wild-type bristle lengths. Moreover, when X/B⁺ brothers of these X/sc⁴sc⁸ descendants are crossed to sc⁴sc⁸/dl-49 females, all of the X/sc⁴sc⁸ daughters are bobbed-plus. Thus, the bobbed-plus phenotype is stable even after one generation over a bᵇ⁺ Y chromosome. In the remaining two cases the X/sc⁴sc⁸
descendants exhibited intermediate bristle lengths. In neither of those two cases were the testcross progeny bobbed by other criteria. As suggested by Locker and Prud'homme (1973; but see DISCUSSION), individuals such as these may represent the appearance of weak bb alleles with low penetrance. However, it is also possible that these two cases may result from the instability of magnified bb+ alleles that has been reported by others (Ritossa 1968a, b; Henderson and Ritossa 1970; but see Locker 1976). Regardless, our data are in agreement with the conclusions of Tartof (1973) that most, if not all, of the wild-type progeny observed in the sc4sc8 assay correspond to stable reversion at the bb locus.

The Ybb− assay produced different results with respect to the heritability of the bobbed-plus phenotype. In the case of the 24 fertile males tested for the Ybb− assay, only 21% (5 of 24) produced X/sc4sc8 progeny with wild-type bristle lengths, 63% (15 of 24) produced descendants with intermediate bristle length as well as slight etching, and 17% (4 of 24) produced X/sc4sc8 descendants that displayed a severe bobbed phenotype. These data are in concordance with the observations of Ritossa (1968a, b) and of Malva, Volpe and Gargiulo (1980) that the majority of the wild-type progeny observed in the early gen-
FIGURE 5.—The mating scheme used to control for the autosomal genome in the Ybb− assay. Cy and Ubx denote the balancers SMI and TM6, respectively.

The mating scheme used to control for the autosomal genome in the Ybb− assay. Cy and Ubx denote the balancers SMI and TM6, respectively.

iterations of the Ybb− assay do not carry stable reversions at the bobbed locus. Indeed, if the frequency of wild types in the Ybb− assay is corrected to account for only bb+ revertants by multiplying 0.47 (the fraction of individuals with wild-type bristle lengths) by 0.21 (the fraction of individuals that testcrossed as true bb+ revertants), the frequency of magnification may be estimated as 0.10. This value is identical to that (0.10) observed for the sc4sc8 assay. Thus, the two assays appear to differ primarily in that the Ybb− assay detects a large number of wild-type, or near wild-type, progeny that do not result from true genetic reversion.

Autosomal modifiers of the bobbed phenotype are revealed by the Ybb− assay: In attempting to understand these discrepancies between the two assays, we noticed that although the sc4sc8 assay consists of an outcross of bb2/BsYbb− males to females from the sc4sca/dZ-#9 stock, the Ybb− assay consists of repetitive backcrosses to females from the C(I)RM,w/B'Ybb− stock. This repetitive backcrossing might explain the behavior of the Ybb− assay if the C(I)RM,w/B'Ybb− stock carried modifiers of bb expression, such that the penetrance of a given bb allele decreased as the autosomal contribution of the C(I)RM,w/B'Ybb− stock increased.

To test for autosomal effects on the expression of the bobbed phenotype, we performed a Ybb− assay using marked autosomes as diagramed in Figure 5. Bristle length histograms for G0 males and for G1 male progeny of the four possible autosomal genotypes are presented in Figure 6. It should be noted that the G0 bb2/Ybb−;Cy/+;Ubx/+ males have a bristle length distribution identical to that observed for G0 bb2/BYbb− males (see Figure 3). Similarly, control bb2/BY;Cy/+;Ubx/+ males are phenotypically identical to control bb2/BY males (data not shown). This demonstrates that neither the Cy nor the Ubx chromosome has a strong effect on the penetrance of bb or bb+. However, it may be seen that G1 males in which all four autosomes are derived from the C(I)RM, y w/Ybb− stock are much less bobbed than either their fathers or their brothers that possess one or more autosomes from the bb2 stock. In fact, if magnification

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AUTOSOMAL MODIFIERS OF BOBBED

FIGURE 6.—Autosomal effects on the bobbed phenotype in the Ybb- assay. The bristle length histograms for G0, bb'/Ybb--;+/Cy;+/Ubx males, X/YY control males and the four classes of G1 males are presented. Autosomal genotypes are schematically diagramed at right. Darkened chromosomes correspond to autosomes derived from the C(I)RM,y w/Ybb- stock.

were measured using only males in which all four autosomes are derived from the C(1)RM,y w/Ybb- stock, it would appear to be a uniform process that results in near complete reversion among all the male progeny. On the other hand, the bristle length histograms for males with one or more paternal autosomes resemble those observed in a sc4sc8 assay. In this experiment we also followed a second characteristic of the bobbed phenotype; namely, abdominal etching. In males that carried all four maternal autosomes, only 6.7% of the progeny were etched, whereas in males that carried one or more paternal autosomes, the frequency of etched males ranged from 23 to 36% [+/Cy; +/ + (36%), +/+; +/Ubx (28%) and +/Cy; +/Ubx (23%)]. This suggests that these autosomal modifiers located on both the second and third chromosomes have a general effect on at least two aspects of the bobbed phenotype. Our data
also demonstrate that neither of the maternal autosomes is itself sufficient. Thus, the amelioration of the bobbed phenotype appears to result from the synergistic action of one or more genes located on both chromosomes.

It should be noted that the females used in this experiment were derived from a stock of $X/Ybb^-$ males crossed to $C(1)RM_y w/Ybb^-$ females, rather than the $C(1)RM_w/B^+Ybb^-$ females used above. Similar results were, however, obtained using females from the $X/B^+Ybb^-$ crossed to $C(1)RM_w/B^5Ybb^-$ stock in a separate experiment (data not shown). These two stocks are of independent origin and have been maintained separately for over 130 generations. Thus, the accumulation of autosomal modifiers may be a general property of stocks of $X/Ybb^-$ males crossed to $C(1)RM/Ybb^-$ females.

Based on these data, we conclude that a large component of the phenotypic reversion observed in the $Ybb^-$ assay is in fact not due to changes at the $bb$ locus, but rather to the accumulation of autosomal modifiers. Indeed, when such modifiers are excluded by the use of marked autosomes, the kinetics of the $sc^4sc^8$ and $Ybb^-$ assays are indistinguishable.

**Two magnification-defective mutants suppress magnification only in the $sc^4sc^8$ assay:** If autosomal modifiers are a major component of the magnification process, then mutants that block true genetic reversion at the bobbed locus would be expected to have differential effects on the two assays for magnification. (i.e., Mutants that block genetic reversion in the $sc^4sc^8$ assay would be expected to slow down magnification in the $Ybb^-$ assay by decreasing the frequency of true revertants. However, due to the modifying loci from the $C(1)RM/Ybb^-$ stock, the accumulation of $bb/Ybb^-$ progeny with intermediate or near wild-type phenotypes should proceed unabated). Repair-defective mutants $mei-41$ and $mus-108$ have previously been shown to be magnification-defective (i.e., to block genetic reversion at the $bb$ locus) in the $sc^4se^8$ assay (Hawley and Tartof 1983; Hawley et al. 1985). $mei-41$ has been shown to decrease only the frequency of premeiotic magnification events, whereas $mus-108$ blocks both meiotic and premeiotic events (Hawley et al. 1985). Polito et al. (1982) have also presented evidence that $mei-9$ inhibits magnification in the $Ybb^-$ assay. Although we find no evidence for an effect of $mei-9$ in either assay, the $mei-41$ and $mus-108$ mutations were shown to affect the $Ybb^-$ assay in the manner predicted.

We first consider the effects of these mutations in the $sc^4se^8$ assay. It may be seen (Figure 2, panels B, C and D) that, although $mei-9 bb^2/B^+Ybb^-$ males produced a significant fraction of individuals with wild-type bristle lengths, $mei-41 bb^2/B^+Ybb^-$ and $mus-108 bb^2/B^+Ybb^-$ males produced very few progeny with wild-type bristle lengths. In fact, the bristle length distributions of $X/sc^4se^8$ progeny from $mei-41 bb^2/B^+Ybb^-$ and $mus-108 bb^2/B^+Ybb^-$ males were not significantly different from those obtained from $mei-41 bb^2/BY$ and $mus-108 bb^2/BY$ (nonmagnifying) males ($t = 0.563, P > 0.5$ for $mus-108$ and $t = 1.43, P > 0.1$ for $mei-41$). Thus, we confirm the observations of Hawley and Tartof (1983) and of Hawley et al. (1985) that magnification in the $sc^4se^8$ assay requires the wild-type products of $mei-41$ and $mus-108$, but not $mei-9$.

Magnification in $mei-41$-, $mus-108$- and $mei-9$-bearing males was also exam-
Autosomal modifiers enhance the frequency of magnification: It has been generally observed that the frequencies of magnification in the $Ybb^-$ assay exceed those observed in the $sc^4sc^8$ assay. Indeed, when studying X chromosomal rDNA magnification using the $Ybb^-$ assay, Ritossa (1968b) has observed nearly 100% magnification (bobbed to bobbed-plus) in a single step. On the other hand, X chromosomal magnification studies using a number of $bb$ alleles in the $sc^4sc^8$ assay (Tartof 1971; Hawley and Tartof 1983, 1985; Hawley et al. 1985) have yielded much lower fractions of $bb^+$ progeny (usually 20% or less). We have demonstrated that these differences in frequency are not a consequence solely of the use of different $bb$ alleles. Rather, when magnification of $bb^2$ was measured in the $sc^4sc^8$ assay only 10% $bb^+$ progeny were observed compared to 47% in the $Ybb^-$ assay. We have shown that this difference is a consequence of the autosomal modifiers present in the $C(1)RM/Ybb^-$ stock. Since we predict that such modifiers are common in stocks in which the males carry any $Ybb^-$ chromosome, we argue that the differences in magnification frequencies observed by previous investigators utilizing different assays may often reflect the action of these modifiers.
Our argument should not be construed to suggest that different \(bb\) alleles have identical frequencies of magnification. Indeed, various \(X\) \(bobbed\) alleles show different frequencies of magnification even when measured by the same assay (TARTOF 1973). Moreover, \(Y\) chromosomal \(bb\) alleles consistently show very high levels of magnification in a variety of genetic assays (BONCINELLI et al. 1972; ATWOOD 1969; RITOSSA 1976; HAWLEY and TARTOF 1983). We are suggesting rather that autosomal modifiers may explain some of the kinetic differences observed when similar or identical \(bb\) alleles are compared by different assays.

The effect of modifiers on the heritability of the bobbed-plus phenotype:

We also argue that these modifiers explain some, but certainly not all, of the observations suggesting that many newly magnified bobbed-plus progeny do not carry heritable and stable \(bb^+\) alleles. Before considering this point further it is necessary to restate the three major tenets of these studies. First, RITOSSA (1968a, b) and others (HENDERSON and RITOSSA 1969; BONCINELLI et al. 1972; LOCKER and PRUD'HOMME 1973; LOCKER 1976) have shown that the \(bb\) loci carried by bobbed-plus progeny recovered after one or two generations of magnification frequently retest as strongly \(bb\) following association with a \(bb^+\) homologue for several generations. LOCKER (1976) has demonstrated that this instability is usually, but not always, confined to those cases where the \(G_1\) individual exhibits incomplete phenotypic reversion; instability was only rarely observed in the descendents of strongly bobbed-plus individuals. Second, RITOSSA (1968a, b) and others (BONCINELLI et al. 1972; HENDERSON and RITOSSA 1969; RITOSSA 1976) have observed that, after many cycles of magnification, most if not all of the bobbed-plus progeny carry stable and heritable \(bb^+\) alleles. Third, \(X\) chromosomal instability is rarely observed when using the \(sc^4sc^8\) assay (TARTOF 1974; HAWLEY and TARTOF 1985).

We have already shown above that a large fraction of the phenotypically bobbed-plus progeny obtained at \(G_1\) in the \(Ybb^-\) assay do not carry a \(bb^+\) revertant locus but, rather, are homozygous for the autosomal modifiers of bobbed. A majority of these males do not retest as \(bb^+\) after even one generation over a \(bb^+\) homologue; at least some of this instability must be due to the dilution of the autosomal modifiers during the outcrossing required to maintain the \(X\) over a \(bb^+\) homologue. The effects of these modifiers can explain the observation that most, if not all, of these bobbed-plus progeny examined after several generations of magnification carry heritable \(bb^+\) alleles: at \(G_2\) the sources of bobbed-plus progeny are (1) sons of \(G_1\) fathers already carrying a \(bb^+\) revertant, (2) \(bb^+\) bearing sons from magnifying \(bb/Ybb^-\) \(G_1\) males and (3) \(bb/Ybb^-\) males made phenotypically bobbed-plus by the modifying loci. Thus, with each generation the fraction of true revertant progeny should increase until, by later generations, the entire population of males should be genotypically \(bb^+/Ybb^-\). This is exactly what is observed. Finally, the lack of instability in the \(sc^4sc^8\) assay can be explained as a consequence of the failure of modifying loci to become homozygous in this assay.

We do not argue, however, that these modifiers can explain all of the instability that has been observed by others. Indeed, LOCKER (1976) has observed
rare reversions of strong $bb^+$ loci to $bb^1$ alleles within a few generations opposite a $bb^+$ balancer chromosome. Such phenomena cannot be a consequence of autosomal modifiers; rather, they provide the substrates for further investigation.

A model that relates the autosomal modifiers to rDNA amplification: Numerous studies of the molecular basis of rDNA magnification have demonstrated large increases in the rDNA redundancy of bobbed males of the genotype $bb/Ybb^-$ (Boncinelli et al. 1972; Ritossa 1976, Locker and Marrakech 1977; De Cicco and Glover 1983). In fact, the rDNA redundancy in these males frequently exceeds that observed in genetically wild-type $bb^+$/Ybb+ males. However, it should be noted that these increases in rDNA redundancy are not restricted to males undergoing magnification. Tartof (1973) has demonstrated that disproportional replication of the rDNA will occur in numerous genotypes including $X/Ybb^-$, $X/O$ and $X/sc^4sc^8$. Indeed, phenotypically bobbed $bb^2/sc^4sc^8$ females also possess more rRNA genes than do $bb^+$/bb+ wild-type females. Tartof (1971, 1973) has termed this process rDNA compensation.

The observations of Locker and Prud'Homme (1973) and Procunier and Tartof (1978), which demonstrate that $In(1)w^{am}$ cannot compensate and also magnifies at low frequencies in both assays, provide an argument in favor of compensation and magnification being related phenomena. Indeed, several authors have suggested that the rDNA amplification observed in $X/Ybb^-$ males may facilitate at least some magnification events (Boncinelli et al. 1972; Ritossa 1976; De Cicco and Glover 1983; Hawley et al. 1985). Like the bobbed phenotype, compensation is also sensitive to the influence of autosomal background. Tartof (1973) has shown that when $bb^2/sc^4sc^8$ females were repeatedly backcrossed to $sc^4sc^8/BsY$ brothers, the amount of compensation increased over each of five generations without resulting in genetic reversion at the $bb$ locus. Moreover, following several generations of outcrossing, the $bb^2$ X chromosome showed normal levels of compensation when crossed again to $sc^4sc^8/dl-49$ females. This suggests that the increase in compensatory ability may be a consequence of modifiers present in the $sc^4sc^8$ stock. Similarly, Dutton and Krider (1984) have demonstrated that wild-type X chromosomes carried by $X/O$ males compensate to different levels in different genetic backgrounds. In an attempt to relate these observations to our own findings, we present below a plausible but highly speculative model relating the effect of modifiers identified here to the phenomena of rDNA compensation.

We propose that these modifiers act by allowing the increase in rDNA redundancy that occurs in both the soma and germlines of $X/Ybb^-$ males (Ritossa et al. 1971) to exert a phenotypic effect. We envision that these modifiers might act by increasing the degree to which the amplified rDNA is expressed. In making this hypothesis, we are simply extending the suggestions of Ritossa et al. (1971) and Tartof (1973) that the amplified rDNA is nonfunctional by suggesting that the modifiers act by allowing this rDNA to be transcribed. Toward this end, we are measuring rDNA abundance and transcription in $bb$/Ybb- males that are either heterozygous or homozygous for the modifying loci.
Autosomal modifiers in terms of models of magnification: As noted, the use of two assays for magnification has led to the construction of two very different models of the magnification process. TARTOF (1973) has proposed that \( bb^1 \) mutations and \( bb^+ \) reversions occur as reciprocal consequences of unequal sister chromatid exchange. This model is based on the absence of magnification in males bearing a ring-X \( bb \) chromosome (TARTOF 1973, 1974), the observation of rDNA reductants among the progeny of magnifying males (LOCKER and PRUD'HOMME 1973; TARTOF 1974) and the equal recovery of magnified and reduced \( bb \) X chromosomes following meiotic magnification events (HAWLEY and TARTOF 1985). The data for \( bb \) to \( bb^+ \) reversion presented here are in no way inconsistent with this model. However, HAWLEY et al. (1985) have suggested that, although unequal sister chromatid exchange represents an excellent paradigm for meiotic magnification events, it may not be the mechanism by which premeiotic magnification events arise.

On the other hand, RITOSSA (1976) has proposed a model in which rDNA excises from the chromosome, replicates extrachromosomally and then attaches to the chromosome in an unstable fashion. This model was developed to explain the apparent frequent instability of \( bb \) reversion, as well as the gradual reduction in the severity of the bobbed phenotype over several generations. The data presented above demonstrate that these phenomena are largely a consequence of the accumulation of autosomal modifiers and not of changes at the \( bb \) locus. Nonetheless, the RITOSSA model, or some variant of it, may be necessary to understand the relationship of compensating events to magnification—particularly premeiotic magnification.

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AUTOSOMAL MODIFIERS OF BOBBED 319


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