ON THE INHERITANCE OF rDNA OF MAGNIFIED BOBBED LOCI IN *D. MELANOGASTER*

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THE bobbed locus in the *X* and *Y* chromosomes of *Drosophila melanogaster* is a compound locus composed of genes for ribosomal RNA (RITOSSA, ATWOOD and SPIEGELMAN 1966). This locus is composed of a variable number of genes which have an additive effect up to a certain threshold (STERN 1929). Single loci, in which the number of genes for 28 and 18 S rRNA is less than 130 are hypomorphic since they produce subnormal effect and constitute a series of bobbed isoalleles. Under normal conditions, 130 such genes are sufficient to guarantee a normal phenotype. In such a case, we call the locus a wild-type member of the isoallelic series.

We have recently discussed some possible reasons for variation of the rDNA content (RITOSSA and SCALA 1969). Variations may be attained by selection of the most appropriate redundancy which is produced by mechanisms such as unequal crossing over (RITOSSA, ATWOOD and SPIEGELMAN 1966; SCHALET 1967). These mechanisms occur with low frequency. Recently, however, cases have been reported in which changes of redundancy can occur very rapidly (RITOSSA 1968b; ATWOOD 1969).

If males are produced which have an extreme bobbed phenotype, their progeny appear wild type even if the sex chromosomes, the carriers of rDNA, are unchanged. This shift to wild type is achieved by rapid accumulation of genes for rRNA, apparently at the bobbed locus itself. Such a bobbed locus, which we call "magnified" bobbed (bb^m), has other peculiar characteristics which are presently under study. When a newly magnified bobbed locus (bb^{m1} or bb^{m2} of Figure 3) is associated with a normal bb^+ locus, it can gradually revert to the original mutated condition. If, instead, a bobbed locus, which underwent the magnification process some generations ago (bb^{m3} , bb^{m4} , etc. of Figure 3), is associated with a normal bb^+ locus, only a small fraction of the progeny reverts to the original, mutated condition. The purpose of this work was to examine the inheritance of rDNA of magnified bobbed loci in this last case. The striking conclusion was reached that the rDNA of magnified bobbed loci is not inherited in an additive way.

MATERIALS AND METHODS

Drosophila stocks: The chromosome $Y^8X \cdot Y^L$, $In(1)EN$, γB , was obtained from Professor COOPER. $Y^8X \cdot Y^L$, $In(1)EN dl-49$, $\gamma v f car/C(1)RM$, $\gamma^2 su(w^a)w^a bb$ was from the University of Rome collection; $g^2 ty bb/C(1)DX$, γf flies were from the Pasadena Collection; Ybb^- was from

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w sn bb⁺ / Ybb⁻ and *C(1)RM, γ v f* obtained from the Philadelphia collection. Wild-type flies used were Canton-S from the Pavia collection. All genetic crosses were made under standard conditions.

DNA/RNA hybridization: DNA was extracted from at least five grams adult *Drosophila* as previously described (RITOSSA, ATWOOD and SPIEGELMAN 1966). RNA was labeled with ³H or ³²P as described previously, and a purified ribosomal fraction was obtained by MAK column chromatography. The specific activity of the RNA was between 20,000 and 60,000 cpm/μg. Hybridization was accomplished using the method of GILLESPIE and SPIEGELMAN (1965), with 30–50 μg DNA per filter. Unannealed rRNA was removed from the filters with RNase digestion (20 μg/ml) for 1 hr at 30°C. Deviations from the mean value percent hybridization were less than 0.02%.

RESULTS

Reference stock: A stock was prepared from a single *Y^SX·Y^L* chromosome so that males contained one such element and females two. The majority of the chromatin of the X is inverted (*Y^S In(1)EN·Y^L*). The attached-XY chromosome is particularly useful for testing whether the rDNA is carried exclusively by the sex chromosomes. It was necessary to be sure that no autosomal rDNA was present in the reference stock. That the autosomes carry no rDNA is illustrated in Figure 1. The rDNA content of males and females is in a ratio of 1 to 2.

Each saturation point was corrected for the amount of DNA which was carried by the stock being considered. The DNA content of the X and Y is estimated to be the same and equivalent to that of one arm of either the second or the third chromosome. Thus there are 10 major arms, each assumed to correspond to 0.1 of the diploid genome; the DNA content of the fourth chromosome is considered negligible. For example, the DNA of a female (that of the reference stock) is estimated

FIGURE 1.—Saturation curves after hybridization of DNA from males and females of reference stock with rRNA. The stock was obtained starting with a single *Y^SX·Y^L* chromosome.

FIGURE 2.—Metaphase chromosomes of females (a) and males (b) of reference stock.

to be 1.2 times that of a normal genome (Figure 2). Corrections are made proportionally for all the data presented in this work. These corrections are made by multiplying the observed hybridization values by the following factors:

Genome	Normalization factor
XX	1
XY	1
XYY	1.1
XXY	1.1
XXYY	1.2
X0	0.9

These normalization factors are only approximations to the actual values.

Average hybridization values for the reference stock are given in Table 1. It is obvious from this table and Figure 1 that in the reference stock: a) all rDNA is carried in the $Y^S X \cdot Y^L$ element within the limits of resolution of the hybridization technique; b) the rDNA is inherited in a regular fashion with this element, and c) the rDNA of the reference chromosome is 0.235% that of the diploid genome.

Inheritance of rDNA of the bb^+ locus: The fact that males and females of the

TABLE 1
rDNA content of reference stocks

Genetic constitution of DNA source	(rRNA/DNA) × 100 at saturation				Average
	I	II	III	IV	
2A + $Y^S X \cdot Y^L$	0.240	0.220	0.250	0.230	0.235
2A + $2Y^S X \cdot Y^L$	0.440	0.480		0.490	0.470
Canton-S bb^+ / bb^+	Average of 10 determinations				0.349
$Y^S X \cdot Y^L / bb^+$	0.418	0.396			0.407

reference stock have saturation levels which are proportional to the number of X·Y elements demonstrates a normal inheritance for DNA through the sex chromosomes. To verify additivity of rDNA in heterozygotes we also combined the reference $Y^S X \cdot Y^L$ chromosome with the bb^+ locus of the X chromosome of Canton-S stock.

Canton-S females were crossed with $Y^S X \cdot Y^L$ males of the reference stock. $bb^+ / Y^S X \cdot Y^L$ daughters were harvested and the saturation levels of their DNA, as well as that of Canton-S females, was determined following hybridization with

FIGURE 3.—Scheme of the crosses to produce bb^m loci. Males of composition $g^2 ty bb / Ybb^+$ are crossed with females $C(1) / Ybb^-$. The males obtained after this cross show a strong bobbed phenotype ($g^2 ty bb / Ybb^-$). When these males are crossed again to females $C(1) / Ybb^-$, males are obtained which are no longer bobbed in phenotype while relevant chromosomes are the same ($g^2 ty bb^m / Ybb^-$).

rRNA. These results are shown in Table 1. The rDNA content per bb^+ locus in the Canton-S strain is 0.175%. Previous estimates gave saturation values ranging between 0.170 and 0.185%. The rDNA content of the bb^+ locus in $bb^+/X \cdot Y$ females may also be calculated as follows: $0.407 - 0.235 = 0.172$. It is apparent from Table 1 that rDNA is perfectly additive.

Preparation and characterization of bb^m : Magnification of the bb locus of the g^2ty bb chromosome was obtained as previously described (RITOSSA 1968b). The scheme of these crosses is repeated in Figure 3. The bb locus is numbered consecutively by generation following the magnification process, e.g., the first generation is bb^{m1} .

One of the relevant facts in the magnification process is the rapid shift of the bobbed phenotype to wild type, which is paralleled by a rapid increase in the amount of rDNA. This accumulation reaches a plateau in bb^{m2} which is maintained in succeeding generations (RITOSSA 1968b). Since the main purpose of this work is to follow the genetic behavior of the rDNA in a magnified locus when it has reached a "steady" rDNA content, the rDNA in males carrying the bb^m locus in a condition of complete magnification is again estimated by means of hybridization. These results are shown in Table 2.

Behavior of the rDNA of bb^m with bb^+ : The magnified bobbed locus is not stable (RITOSSA 1968b). If bb^{m1} or bb^{m2} are associated with a bb^+ locus, a progressive reversion to the bobbed phenotype is observed (Figure 5). If, instead a bb^{m3} or bb^{m4} locus, in which a "stable" rDNA content is observed, is associated with a normal bb^+ locus, only a small fraction of the progeny reverts to the original mutated phenotype.

The experimental plan we followed to study this behavior is shown in Figure 4. It consists of introducing the bb^m locus of known rDNA content into females of the constitution $bb^m/Y^sX \cdot Y^L$ for successive generations.

g^2ty bb^m/Ybb^- males are crossed to females of our reference stock, homozygous for the $Y^sX \cdot Y^L$ chromosome. F_1 females ($bb^m/Y^sX \cdot Y^L$) are then crossed with $Y^sX \cdot Y^L$ males and so on as illustrated in the figure. Eventual phenotypic rever-

TABLE 2
rRNA/DNA ratios obtained with DNA from flies bb^m/Ybb^-
as compared with $bb^m/0$ individuals

Genetic constitution of DNA source	(rRNA/DNA) $\times$ 100 at saturation	X contribution (%) assuming $Ybb^- = 0.08\%$
g^2ty bb^{m3}/Ybb^-	0.25*	0.17
g^2ty bb^{m4}/Ybb^-	0.25*	0.17
g^2ty bb^{m5}/Ybb^-	0.25*	0.17
g^2ty bb^{m6}/Ybb^-	0.23*	0.15
g^2ty $bb^{m3}/0$	0.25†	0.25
g^2ty $bb^{m10}/0$	0.26†	0.26

* Average of three determinations

† Obtained from RITOSSA (1968b)

FIGURE 4.—Scheme of the crosses made to study the inheritance of rDNA of bb^m loci when associated with a bb^+ locus. $g^2 ty bb^m/Ybb^-$ males are crossed to $Y^S X \cdot Y^L/Y^S X \cdot Y^L$ females. The X carried by the $Y^S X \cdot Y^L$ chromosome is inverted ($In(1)EN$) and carries γ and B . γ is included in the inversion and practically cannot be removed from the $Y^S X \cdot Y^L$ chromosome. F_1 females, which have Bar eye, are then crossed with $X^S X \cdot Y^L$ males. Sons are obtained which are of two types, as far as body color is considered. γ^+ ones, which carry the bb^m locus (males (c) of the figure), are of the $X/0$ type and are scored for reversion of the bb^m locus to the original expressivity. In some cases they have also been used for rRNA/DNA hybridization (Table 5). Yellow sons (males (b) of the figure) have the $Y^S X \cdot Y^L$ chromosome and are counted to monitor possible abnormalities of segregation and for formation of bb isoalleles (none was found in our studies). Daughters are also obtained which are either γ or γ^+ . Yellow daughters (females (a) of the figure) are homozygous for the reference chromosome and are counted for reference. γ^+ ones (females (d) of the figure) are of the $bb^m/Y^S X \cdot Y^L$ composition. They are harvested for DNA extraction and some are mated with $Y^S X \cdot Y^L$ males. Crosses are continued for many generations and the same analysis of the progeny is made as described here.

sion is observed scoring γ^+ males (which are of the $X/0$ type (see legend to Figure 4). rDNA content of bb^m was followed by hybridization with rRNA in this female line.

Figure 5 shows that only few $g^2 ty bb^{m4}/0$ and $g^2 ty bb^{m7}/0$ males return to the original bb phenotype even after many generations of association with the $Y^S X \cdot Y^L$

FIGURE 5.—Percentage of individuals maintaining the magnified condition after the bb^m locus was associated with a bb^+ locus for a different number of generations. $g^2 ty bb^m/Ybb^-$ males of different generation time after magnification (bb^{m2} , bb^{m4} , bb^{m7} of Figure 3), were crossed to reference females as indicated in Figure 4. $bb^m/0$ males (males(c) of Figure 4) were scored for bb phenotype after the bb^m locus was associated with the bb^+ locus of reference chromosome for an increasing number of generations, as shown in the abscissa. Etched abdomen was used as a parameter for bb phenotype. (Filled circles refer to bb^{m7} , open circles refer to bb^{m4} , half-filled circles refer to bb^{m2} .)

chromosome. On the contrary, the bb^{m2} locus shows a striking reversion in similar conditions.

By hybridization, the bb^{m3} locus has been followed for 3 generations of combination with the $Y^S X \cdot Y^L$ chromosome. The bb^{m5} locus was determined in the first generation. The relevant data are given in Table 3. Since it is estimated that the bb^{m3} locus carries 0.170% rDNA, it is clear that in the first generation with the $Y^S X \cdot Y^L$ chromosome, if the rDNA content of this element is assumed constant, there is a substantial loss of rDNA. The same is true for the bb^{m5} locus. No detectable loss is seen in the succeeding generations.

The problem of using the Ybb^- chromosome: Table 2 shows that the rDNA content of $g^2 ty bb^m/Ybb^-$ males we have calculated is of the same order of magnitude as that previously obtained from $g^2 ty bb^m/0$ males (Rirrossa 1968b). However, it was also previously reported that the Ybb^- chromosome carries some non-operative rDNA (Rirrossa 1968a). The percentage of rDNA carried by the Ybb^- chromosome (as referred to the diploid genome) was estimated to be between 0.07 and 0.1% with a mean value of 0.09%. Hence we are apparently faced with a problem of the nonadditivity of rDNA here also.

To clarify this point, $g^2 ty bb^{m3}/Ybb^-$ males were crossed with $C(1)RM/0$ females and $g^2 ty bb^{m3}/0$ males were obtained. The saturation values of DNA from these males, after hybridization with rRNA are given in Table 4. These results

TABLE 3

rDNA content of bb^m coupled with bb⁺ in bb^m/Y^{SX}·Y^L females

<i>bb</i> allele	Number of generations in combination with Y ^{SX} ·Y ^L	(rRNA/DNA) × 100	Y ^{SX} ·Y ^L contribution	<i>bb^m</i> contribution
<i>bb^{m3}</i>	1	0.352	0.235	0.117
	2	0.363	0.235	0.128
	3	0.360	0.235	0.125
<i>bb^{m5}</i>	1	0.341	0.235	0.106

TABLE 4

rRNA/DNA ratios with DNA from bb^m/0 and Y^{SX}·Y^L/Ybb⁻ males

Genetic constitution of DNA source	(rRNA/DNA) × 100 at saturation
<i>g² ty bb^{m3}/0</i>	0.23†
<i>Y^{SX}·Y^L/Ybb⁻</i>	0.31*

* Assuming Y^{SX}·Y^L contribution is 0.23%, Ybb⁻ contribution is 0.08%.

† Average of three determinations.

confirm previous estimates of saturation levels obtained with *g²ty bb^m/0* males, but raise doubts that the *Ybb⁻* carries rDNA. This point was further tested using the reference *X·Y* chromosome. *g²ty bb^{m3}/Ybb⁻* males were crossed to *Y^{SX}·Y^L/Y^{SX}·Y^L* females. *Y^{SX}·Y^L/Ybb⁻* sons were harvested and saturation levels were measured after hybridization of their DNA with ribosomal RNA. These results are reported in Table 4 and confirm that, in this combination, the *Ybb⁻* chromosome contains about 0.08% rDNA, which is close to previous estimates.

Behavior of bb^m when placed with Y^{SX}·Y^L and returned to the X/0 condition: It is apparent from Table 3 that when *bb^m* is associated with the *Y^{SX}·Y^L* chromosome, there is a loss of additivity of rDNA. A cross was then made in which the *bb^m* locus after this association was again placed in males of *X/0* composition. This cross is illustrated in Figure 4. *g²ty bb^{m3}/Y^{SX}·Y^L*, *γ⁺B* females (both from G₁ and G₂ of Figure 4) were crossed with *Y^{SX}·Y^L* males, as illustrated in the same figure. Sterile males of composition *g² ty bb^m/0* were collected and the rDNA analyzed by hybridization to rRNA. The corrected saturation values after these experiments are given in Table 5. These values are of the same order of magni-

TABLE 5

*rRNA/DNA ratios of bb^m/0 males obtained by crossing
bb^{m3}/Y^{SX}·Y^L females with Y^{SX}·Y^L males*

Number of generations of association of <i>bb^{m3}</i> with Y ^{SX} ·Y ^L	(rRNA/DNA) × 100 in <i>bb^{m3}/0</i> males
1	0.23
	0.22
2	0.22
	0.23

tude as those obtained for the corresponding bb^m locus before it had been present in females of the type $bb^m/Y^sX \cdot Y^L$ (Table 4). This is in spite of the fact that a minority of these males showed a bobbed phenotype. The data were obtained using the entire progeny.

DISCUSSION

The ability of a strong bobbed isoallele to revert to a wild phenotype in a few generations has been reported previously (Ritossa 1968b; Atwood 1969; Ritossa and Scala 1969). This rapid reversion, which we call magnification of bb (bb^m), was shown to involve a rapid accumulation of rDNA up to a level which is afterward maintained in suitable chromosomal compositions. The bb^m can revert to the original expression if placed in combination with a bb^+ allele.

The instability of bb^m , defined as the ability to revert to the original bb expressivity, is very apparent only in newly formed bb^m loci (bb^{m1} and bb^{m2} of Figure 3). The instability is not so apparent if bb^m loci are used which reached a constant rDNA level (bb^{m3} etc.). The inheritance of the rDNA of such bb^m loci was followed after they were associated with a bb^+ locus in a female line. We established in the reference stock that within the resolution limits of the hybridization technique all the rDNA was associated with the sex chromosomes and that the amount of rDNA per single $Y^sX \cdot Y^L$ chromosome was 0.235% of the diploid genome of the fly. Additivity of rDNA was also shown when the bb^+ locus of the X chromosome of Canton-S stock was associated with the $Y^sX \cdot Y^L$ chromosome of our reference stock (Table 1), thus excluding the existence of an optimum of rDNA per fly which is achieved independently of the rDNA carried by the sex chromosomes of the parents. There are different stocks of *Drosophila* which have characteristic rDNA redundancy that is maintained, generation after generation, with only minor changes (Ritossa and Scala 1969). The rDNA content of magnified bobbed loci, on the contrary, appears not to be constant. We found nonadditivity of rDNA when bb^m was associated with either the Ybb^- or the $Y^sX \cdot Y^L$ chromosomes. Conversely, when the Ybb^- and $Y^sX \cdot Y^L$ chromosomes are included in the same genotype, there is additivity of their rDNA contents (Table 4). Additivity is also observed when they are associated with other chromosomes of known rDNA content (Table 1 of this paper and Figure 3 in Ritossa 1968a).

As a working hypothesis we assume that the nonadditivity of rDNA where the bb^m locus is involved, may be attributable to the bb^m locus itself.

In the cases studied, the rDNA content of the bb^m locus can be estimated as either 0.17 or 0.23% depending on the combination in which bb^m is present; $g^2 ty b^m/Ybb^-$ or $g^2 ty bb^m/0$ (Tables 2 and 3). However, the rDNA of bb^m falls to an average value of 0.12% when associated with the $Y^sX \cdot Y^L$ chromosome which carries 0.235% of the total DNA as rDNA. If males of the sex chromosomal constitution $g^2 ty bb^m/Ybb^-$ (generation n) are crossed with $C(1)/Ybb^-$ females, males are produced of identical genetic constitution, $g^2 ty bb^m/Ybb^-$ (generation n + 1). As shown in Table 2, no drop of rDNA occurs. If the same males (generation n) are crossed to $Y^sX \cdot Y^L/Y^sX \cdot Y^L$ females, the $g^2 ty bb^m/Y^sX \cdot Y^L$ females have an rDNA content which is less than the sum of the rDNA carried by the bb

loci as estimated from the parent cell chromosomes. Because the rDNA of the $Y^sX \cdot Y^L$ element is constant from generation to generation, we are inclined to think that it is the rDNA of the bb^m locus which is lost in such a combination. If this is so, since the sperm of males $g^2 ty bb^m/Ybb^-$ (generation n) is the same for the formation of males $g^2 ty bb^m/Ybb^-$ (generation $n + 1$), or for that of females $g^2 ty bb^m/Y^sX \cdot Y^L$, it is obvious that the loss of rDNA from bb^m in this last combination does not reflect a change which occurred at sperm production in the male parents carrying bb^m , but rather represents a somatic reduction of rDNA from bb^m when this locus is in combination with our reference element. As an alternative, one might postulate that males $g^2 ty bb^m/Ybb^-$ produce sperm with variable rDNA content, those sperm with the proper redundancy being selected later.

It is difficult, however, to visualize how this sperm selection could take place. It is known that the rDNA content does not influence sperm viability (bb^l -bearing or bb -deficient sperm are as viable as bb^+ -bearing ones). On the other hand, if selection operates after fertilization, one should expect altered sex ratios. This is not observed, therefore the most probable mechanism for the loss of rDNA from bb^m in females of the composition $g^2 ty bb^m/Y^sX \cdot Y^L$ is a somatic one occurring after fertilization. The rDNA content of such females, at the first generation, was estimated in two cases to be 0.352 and 0.341%. Theoretical values, if bb^m were inherited in an unaltered state, should have been (according to the estimations of the rDNA content of bb^m in bb^m/Ybb^- and $bb^m/0$ males) either $0.235 + 0.172 = 0.407\%$, or $0.235 + 0.225 = 0.460\%$. One might postulate that such levels of redundancy are unfavorable to the cell and that cells in which a reduction has occurred (by somatic unequal crossing over or similar mechanisms) are preferentially selected for levels of the order of 0.35%. We have shown, however, that females can be produced with redundancy values of exactly this order of magnitude in which no reduction occurs. For example, the genotype $bb^+/Y^sX \cdot Y^L$ gives a hybridization value of 0.418% and $Y^sX \cdot Y^L/Y^sX \cdot Y^L$ gives a value of 0.47%. Hence we can conclude that the rDNA content of the bb^m locus can be conditioned by the type of partner with which it is associated.

The most appealing hypothesis is that the rDNA content of the partner locus determines the rDNA content of the bb^m locus, for the following reasons: (a) The bb^m locus, which has an rDNA content of about 0.12% in combination with the $Y^sX \cdot Y^L$ element, has 0.225% rDNA content when $X/0$ males are produced with the same chromosome. (b) The amount of rDNA in the bb^m is conditioned by the presence or absence of the Ybb^- chromosome, and it is known that this chromosome carries 0.07–0.09% rDNA, even though it is apparently not operative.

It is apparent that the magnified condition of the bobbed locus does not imply a characteristic amount of rDNA. Rather, it would seem that this condition implies the possibility of making, under certain conditions, a given amount of rDNA, i.e., this potentiality depends upon the presence of other elements.

Why a magnified bobbed locus loses the ability to revert to the original expressivity after some generations in the magnified condition is unexplained, as are also the molecular events characterizing the phenomenon, with the exception of these first quantitative efforts.

We regret being unable, at present, to present more comprehensive results. What we are doing is a kind of rough anatomy of the problem, which is indeed complex, but we are pursuing the idea because we think mechanisms of this sort might be operating in other gene systems.

Bobbed, being hypomorphic (MULLER 1932), has been very favorable for the detection of this phenomenon. Amorphic mutations would not lead to its detection. Mechanisms of this sort might be responsible for the production of genetic redundancy and possibly, after stabilization, for the formation of complex loci.

SUMMARY

The inheritance of DNA complementary to ribosomal RNA (rDNA) produced after the magnification process of bobbed isoalleles was studied. It was found that when bb^m is associated with a bb^+ locus the rDNA content is not additive. On the contrary, perfect additivity is observed when normal bb^+ isoalleles are associated together.

LITERATURE CITED

- ATWOOD, K. C., 1969 Some aspects of the bobbed problem in *Drosophila*. *Genetics* **61** (Suppl. No. 1, part 2): 319-327.
- GILLESPIE, D. and S. SPIEGELMAN, 1965 A quantitative assay for DNA-RNA hybrids with DNA immobilized on a membrane. *J. Mol. Biol.* **12**: 829-842.
- MULLER, H. J., 1932 Further studies on the nature and causes of gene mutation. *Proc. 6th Intern. Congr. Genet.* **1**: 213-255.
- RITOSSA, F. M., 1968a Nonoperative DNA complementary to ribosomal RNA. *Proc. Natl. Acad. Sci. U.S.* **59**: 1124-1131. —, 1968b 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. M. and G. SCALA, 1969 Equilibrium variations in the redundancy of rDNA in *Drosophila melanogaster*. *Genetics* **61** (Suppl. No. 1, part 2): 305-317.
- SCHALET, A., 1967 The generation of a variety of alleles associated with exchange within the bobbed locus of *Drosophila melanogaster*. (Abstr.). *Genetics* **56** (Suppl. No. 3, part 2): 587.
- STERN, C., 1929 Ueber die Wirkung multipler Allele. *Biol. Zbl.* **49**: 261-290.