GENETICALLY INDUCED MITOTIC EXCHANGE IN THE HETEROCHROMATIN OF *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

Multiple copies of the 18S and 28S ribosomal RNA cistrons are present in both the *X* and *Y* chromosomes of *Drosophila melanogaster*. Data are presented here that identify a locus, *Rex*, that causes exchange-like events between duplicated ribosomal complexes at the ends of an attached-*XY* chromosome. *Rex*: (1) is close to or in the basal heterochromatin of the *X* chromosome; (2) is semidominant and (its effect) is temperature sensitive; (3) acts maternally; and (4) affects behavior of paternally derived attached-*XY* chromosomes shortly after fertilization. Though, at this point, the existence of *Rex* is known only from its effects on behavior of a particular compound chromosome, it presents intriguing possibilities for understanding regulation of chromosome behavior and organization of the ribosomal cistrons.

ATTACHED-*XY* chromosomes generally behave as stable elements, both meiotically and mitotically. Unexpected production of free *Y* chromosomes had been noted in crosses done for other purposes (ROBBINS 1977). As will be demonstrated in the following, the observed instability of the attached *XY* is not an intrinsic property of the compound chromosome; it is caused by a genetic element located near the centromere of the *X* chromosome.

Three properties of this phenomenon have been examined: (1) the identity and location of the element responsible for instability of the attached *XY*; (2) the mode of action of the variant; and (3) the genetic character of the free *Y* chromosome products. The results are consistent with the hypothesis that attached-*XY* chromosomes introduced into eggs of females heterozygous or homozygous for the detachment-inducing element undergo an exchange-like event between their duplicated ribosomal complexes during the first embryonic mitosis. The detachment-inducing element is here given the name *Rex* as a mnemonic for a dominant inducer of ribosomal gene exchange.

CROSSES AND RESULTS

Except as noted, descriptions of markers and chromosomes used may be found in LINDSLEY and GRELL (1968). All crosses were done on cornmeal, molasses, brewers' yeast medium at 25°, except as noted.

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The attached-*XY* chromosome used was constructed by LINDSLEY as a recombinant between the proximal heterochromatin of $Y^SX\cdot$, *In(1)EN* and y^+Y. It should therefore have the structure $Y^SX\cdot Y^L$, *In(1)EN* with y^+ appended to the tip of Y^L. The *X*-chromosome segment is marked by *y*, *v*, *f* and *B*. Since there have been instances of rearrangement in stocks of this chromosome (T. KAUFMANN, personal communication), the structure of this particular chromosome was examined. Four structural properties were checked: the order of the euchromatin, the positions of the short- and long-arm groups of fertility factors, the position of the y^+ duplication and the bobbed constitution of each end.

The order of the euchromatin was determined from male progeny produced by the cross: y^2 *car*/*XY*, *y v f B*·$y^+ \times$ *y w*/*Y*. Single crossovers occurred throughout the length of the *X* chromosome and, in a sample of 189 males, the tip to centromere distance was 74 map units. Thus, the chromosome is in normal rather than inverted sequence.

To determine the locations of the Y^L and Y^S fertility factors and of y^+, balanced stocks of five *y* and five y^+single crossovers were established from attached *XY*/$y^2 \times$ *FM7Y* crosses. Females from each stock were crossed to $X\cdot Y^S/Y^L$ *and* $X\cdot Y^L/Y^S$ males. The presence of Y^L or Y^S fertility factors was determined by testing non-*FM7* male offspring for fertility. All *y* single-crossover chromosomes were fertile in combination with Y^L but not Y^S, and all y^+ single crossovers were fertile in combination with Y^S but not Y^L. Thus, the arrangement of the *XY* is $Y^SX\cdot Y^L$, *y v f B*·y^+.

The bobbed constitution was also examined by separating the Y^S and Y^L ends of the attached *XY*, except that the recombinants were recovered as crossovers between the attached *XY* and $Df(1)bb^{l158}$, so that the Y^SX derivatives do not carry ribosomal cistrons in the *X* heterochromatin. Free-*Y*-chromosome-bearing crossover males were crossed to *C(1)RM*, y^2 *su*(w^a) w^a/*O* females. In the case of the $X\cdot Y^L$ recombinants, 472 *C(1)RM*/*Y* females and 524 $X\cdot Y^L$/*O* males were recovered. Furthermore, the males showed no thinning of bristles nor any tergite abnormalities. Thus, the attached-*XY* chromosome carries sufficient ribosomal cistrons at the Y^L end to give a bb^+ phenotype. The position of this bb^+ region with respect to the centromere has not been determined, but, from the manner of construction of the *XY*, it is probably on the *X* rather thanY^L side. Crosses of $Y^SX\cdot$, *Df(1)* bb^{l158}/*Y* males to *C(1)RM*/*O* females produced 367 females and 294 males. Of the males, 105 had a *bb* phenotype. Thus, although the Y^S segment of the *XY* carries a sufficient number of ribosomal cistrons for near normal viability, it is probably not a complete set. Variation in *bb* is considered further in a later section.

Identification and localization of Rex: The *Rex* phenotype is characterized by production of free *Y* chromosomes in the offspring of *Rex*-bearing females crossed to males carrying the attached-*XY* chromosome. If both *X* chromosomes of the mother are marked by mutant alleles of *y*, the exceptional products are readily detected as wild-type males. Routine crosses demonstrate that the chromosomes of the exceptional males are a normal, maternally-derived *X* chromosome and a free *Y* chromosome marked by y^+. Since the exceptional males must be derived

from what would have been *X/XY* zygotes, the frequency of detachment is calculated as detachment males/(normal females + detachment males). *Rex* activity was first noted in a chromosome that also carried a lethal deficiency. When the *Rex* female is heterozygous for that chromosome, the number of detachment-bearing males is doubled to account for lethality of the deficiency/y^+Y genotype. Some of the detachment products are gynandromorphs mosaic for X/y^+Y male and phenotypically normal female tissue.

Rex must be located on the *X* chromosome because it segregates from the *X* chromosome balancer *FM7* (MERRIAM 1968). These results are shown in Table 1. To determine the location within the *X* chromosome, individual crossover chromosomes were stocked and tested for detachment-inducing activity. For each recombinant, five to ten *CO/y* females were tested, with the results shown in Table 2. None of the crossovers between *f* and *car* separate *Rex* from the centromere, and 75 out of 76 crossovers between *car* and the centromere were distal to *Rex*. These results place *Rex* in or near the basal heterochromatin of the *X* chromosome.

The mode of action of Rex: The *Rex* phenotype is manifest in crosses where the mother carries *Rex*; yet, the chromosome that breaks down is transmitted from the father. The detachment event must therefore occur in the zygote rather than in the gametes. Does detachment occur because *Rex* is present in the mother, or do detachments occur only in those offspring that receive *Rex*? Several modes of action are possible: detachments might occur only if the *Rex*-bearing base of the chromosome is present in the zygote, *Rex* could transmit some set of modified *X* chromosomes to the affected zygotes, *Rex* could itself be an occasionally mobile element transmitted either stably or cytoplasmically to the few progeny in which detachments occur, or the *Rex* effect could be maternal.

The first two possibilities were examined by scoring *X*-linked markers recovered in detachment-bearing and normal offspring of *Rex* heterozygous females. As shown in Table 2, there is no preferential recovery of either *X* chromosome base. As shown in Table 3, there is no distortion of meiotic recombination evident in either the normal or detachment-bearing offspring of *y cv v f Rex/y* females.

TABLE 1

Sex linkage of the detachment-inducing locus

F_1 female tested	Regular Females	Regular Males	Detachments Complete	Detachments Mosaic	Frequency
Parental generation = $\frac{Df(1)w^{rJ1}, y^2\ sn^3}{FM7} \times y/Y$					
$\frac{Df(1)w^{rJ1}}{y}$	6270	5230	42	7	0.015
$\frac{FM7}{y}$	5420	5229	1	0	0.000

F_1 females of the indicated genotypes were crossed to $Y^SX \cdot Y^L$, $y\ v\ f\ B \cdot y+/O$; spa^{pol}/spa^{pol} males. Detachments were detected by recovery of wild-type males and gynandromorphs having wild-type male tissue.

TABLE 2

Mapping Rex

Parent	Recombinant selected	*Rex* activity	Number of recombinants recovered	Regular Females	Regular Males	Detachments Whole-body *f*+	Detachments Whole-body *f*	Detachments Mosaic	Frequency
$\frac{y^2\ Df(1)w^{rJ1}\ Rex}{y\ cv\ v\ f\ car}$	$y\ cv\ v\ f$	yes	47	19,037	24,980	189	162	70	0.022
		no	0	—	—	—	—	—	—
						car+	*car*		
$\frac{y\ cv\ v\ f\ Rex}{Dp(1;1)sc^{V1},\ y^2\ car{\cdot}y^+}$	$(y\ cv\ v\ f)\ car$*	yes	75	38,533	62,582	488	494	152	0.029
		no†	1	1,440	2,219	0	0	0	0.000

* Recombinants tested were all the *car* chromosomes that were not y^+.

† Includes results of re-tests of this chromosome. Forty detachment products would have been expected if this chromosome had *Rex* activity.

Recombinant-bearing X/Y males were crossed to $C(1)DX,\ y\ f/Y$ females to establish stocks. Males were crossed to y/y females to generate recombinant/y females. These were then crossed to $Y^S X{\cdot}Y^L,\ y\ v\ f\ B{\cdot}y^+/O$ males to test for *Rex* activity.

TABLE 3

X *chromosomes of regular and detachment-bearing males*

$$\frac{y\ cv\ I\ v\ II\ f\ Rex}{y} \times \frac{Y^{S}X{\cdot}Y^{L}, y\ v\ f\ B{\cdot}y^{+}}{O}$$

Offspring scored	Noncrossover f^+	Noncrossover f	Single crossover I f^+	Single crossover I f	Single crossover II f^+	Single crossover II f	Double crossover f^+	Double crossover f
Normal	1059	901	384	417	315	311	45	47
Detachment	112	99	41	32	32	27	4	4

Contingency tests: all classes: $\chi^2 = 3.97$, 7 d.f.
f^+ *vs.* f only: $\chi^2 = 0.50$, 1 d.f.
Regular males from a sample of vials and detachment-bearing males from all vials of the tests of *y cv v f Rex* chromosomes shown in Table 2 were scored for *cv*, *v* and *f*.

The possibility that *Rex* might be stably transmitted independently of the *X* chromosome to the few zygotes in which detachments occur was tested by stocking the *X* chromosomes of detachment-bearing offspring of *Rex* heterozygotes and testing those chromosomes for *Rex* activity. As shown in Table 4a, *Rex* is not preferentially recovered in detachment bearing offspring. The possibility of unstable nonchromosomal transmission was examined in similar fashion, except that the *X* chromosomes were tested immediately after recovery rather than after being stocked. These results are shown in Table 4b and again indicate Mendelian transmission.

It may be concluded from these experiments that detachments occur even when *Rex* is not present in the affected zygote. Thus, the effect of *Rex* is necessarily maternal.

The possibility of an effect of *Rex* on meiotic recombination was examined, with the results as shown in Table 5. Though the difference in recombination frequency between *Rex* and control females is significant, it is small, has not been mapped to *Rex*, and could be an effect of differences in genetic background. In any case, *Rex* assuredly does not increase meiotic recombination in the heterochromatin.

TABLE 4

Rex *activity in the* X *chromosomes of detachment males*

Experiment	*X* chromosome marker	Detachment inducing activity *Rex*	Detachment inducing activity non-*Rex*
a:	f^+	3	32
	f	23	9
b:	car^+	0	10
	car	9	1

Females heterozygous for *Rex* and either *f* or *car* were crossed to *XY/O* males and detachment-bearing males were collected.
a: 35 f^+ and 32 *f X* chromosomes from detachment males were balanced by *FM7* and then tested in *X/y* females for *Rex* activity. Ten females were tested for each chromosome.
b: Ten car^+ and 10 *car* detachment-bearing males were crossed to *y/y* females and *X/y* offspring were tested for *Rex* activity. Ten females were tested for each chromosome.

TABLE 5

Meiotic recombination in Rex *and control females*

Chromosome tested	Female progeny		Male progeny*															
			Noncrossover	Single crossover					Double crossover									
				1	2	3	4	5	1,2	1,3	1,4	1,5	2,3	2,4	2,5	3,4	3,5	4,5
+	2698	y and y^2 males	589	73	223	240	77	48	3	16	1	4	10	4	4	0	0	0
		$y+$ males	665	—	160	220	62	38	—	—	—	—	14	14	6	5	6	0
Rex	2668	y and y^2 males	540	76	226	241	54	31	5	23	8	5	19	13	5	5	1	0
		$y+$ males	651	—	175	222	44	39	—	—	—	—	18	9	10	4	4	6†

Chromosome tested	Map distance					
	1	2	3	4	5	Sum
+	7.5	17.6	20.6	6.6	4.3	56.6
Rex	9.3	19.8	22.1	5.9	4.2	61.3

* Regions indicated are: 1: y to *cv*, 2: *cv* to *v*, 3: *v* to *f*, 4: *f* to *car* and 5: *car* to $y+$.

† Nondisjunction will yield males of the same phenotype. These males have nevertheless been included as double crossovers in calculating map distance.

y cv v f/Dp(1;1)sc^{v1}, y^2 *car*·$y+$ (+) and *y cv v f Rex/Dp(1;1)sc*^{v1}, y^2 *car*·$y+$ (*Rex*) females were crossed to wild-type (Oregon-R) males. Crossovers in region 1 cannot be distinguished in $y+$ male offspring and only the y and y^2 males were used to calculate map distance for that interval. All males were used to calculate the other distances.

In the foregoing presentation, the effects of *Rex* have been considered in a qualitative manner. The tabular data, however, reveal marked variation in detachment frequency from experiment to experiment. One possible source of variation has been probed by testing effects of temperature. *Rex* heterozygous females were reared at 19°, 25° and 29°, and, in each case, they were crossed to *XY/O* males at each of the three temperatures. The data are listed in Table 6a and summarized in Table 6b. While there are other statistically significant, but evidently unsystematic differences in these results, there is a large and consistent change in detachment frequency when egg laying and zygotic development is at 19°, as opposed to 25° or 29°. This temperature effect could be maternal, occurring shortly before egg deposition, or it could be an effect on the detachment process itself.

The dominance of *Rex* was examined in the experiments shown in Table 7. The frequency of detachments produced by heterozygotes and homozygotes differs by about a factor of two in both experiments, but the conclusion that *Rex* is semi-dominant must be tempered by the variability of detachment frequency seen in other experiments. There is no effect of parental origin on *Rex* activity ($\chi^2 = 0.28$ with 1 d.f. for a homogeneity test using the numbers of regular females and detachment-bearing offspring).

The detachment event and its products: A hypothetical origin of free y^+ *Y* detachment chromosomes is schematically illustrated in Figure 1. Though conjectural, it has proven to be a useful heuristic device and is consistent with all of the observations described in the following. This model supposes that detachment

TABLE 6

The effect of temperature on detachment production

a: DATA

Maternal temperature	Zygotic temperature	Regular Females	Regular Males	Detachments Complete	Detachments Mosaic
19°	19°	3659	2887	1	2
19°	25°	730	687	21	1
19°	29°	1520	1279	15	1
25°	19°	1947	1758	1	0
25°	25°	305	457	8	2
25°	29°	581	550	12	0
29°	19°	1458	1339	3	0
29°	25°	257	319	14	3
29°	29°	300	266	7	0

b: FREQUENCIES

Zygotic temperature	Maternal temperature 19°	25°	29°	Mean
19°	0.002	0.001	0.004	0.002
25°	0.057	0.062	0.117	0.079
29°	0.021	0.040	0.045	0.035
Mean	0.027	0.034	0.055	0.039

Df(1)w^{rJ1}, *Rex/w*$^+$*Y* males were crossed to *y/y* females and their daughters were collected at the indicated maternal temperature. The *Df(1)w*^{rJ1}, *Rex/y* females were crossed to $Y^{S}X{\cdot}Y^{L}$, *y v f B*·y^+*/O* males at the indicated zygotic temperature.

TABLE 7

Dominance of Rex

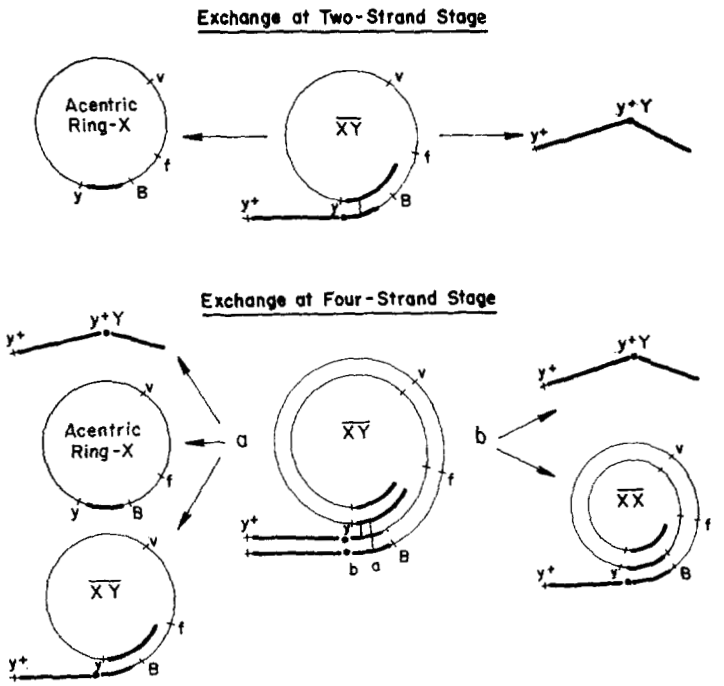
Female tested	Regular Females	Regular Males	Detachments Complete	Detachments Mosaic	Frequency
y cv v f Rex/y	4212	5642	48	6	0.013
y cv v f Rex/y cv v f Rex	2246	2369	54	6	0.026
y w/y w	2147	3354	0	0	0.000
y w car Rex(P)*/y w*	1705	2670	13	0	0.008
y w car Rex(M)*/y w*	1945	2604	12	0	0.006
y w car Rex/y w car Rex	2827	4008	41	4	0.016

Females as indicated were crossed to $Y^{S}X{\cdot}Y^{L}$, *y v f B*$\cdot y^{+}/O$ males. (P) and (M) indicate paternally and maternally transmitted chromosomes. The frequency of detachment is calculated as detachments/(regular females + detachments).

occurs as an exchange-like event between duplicated heterochromatic segments. Other pairing configurations could be drawn, but the products that would result would not have been detected in these crosses so that they are not considered here.

Since *Rex* acts maternally and the attached-*XY* chromosome is paternally derived, the exchange must be mitotic. Depending on the state of replication and the time at which the exchange occurs, both whole-body and mosaic detachment-bearing offspring could be produced. Interpretation of the observations depends in part on the fate of *XXX:XY* mosaics. If metafemale clones survive, most exchanges must occur prior to first embryonic replication since the majority of detachment products are whole-body y^{+} B^{+} males. If metafemale clones can not contribute to the adult, but embryos derived from only the other nucleus survive, four-strand events could also generate y^{+} B^{+} males. Two considerations argue against the latter possibility. First, metafemale:female mosaics can be produced by ring loss, and in such mosaics half the cells are metafemale (SHÜPBACH, WIESCHAUS and NÖTHIGER 1978). It seems *a priori* unlikely that metafemale cells would all die in *XXX:XY* mosaics when they all survive in *XXX:XX* mosaics. Second, gynandromorphs are only a minority (10 to 15%) of detachment products. If the whole-body products were produced by exchanges at the four-strand stage, yet another unlikely assumption would be required. One would have to assume either that offspring in which the *XXX* clone dies are far more viable than *XY:XXY* gynandromorphs, or that one type of exchange (b) is several times more frequent than the other (a). Thus, from both the survival of metafemale cells in *XXX:XX* mosaics and the observed occurrence of many more whole-body than gynandromorph detachment-bearing offspring, it seems likely that the detachment event occurs at a two-strand stage (G1 whole chromatid exchanges) rather than at a four-strand stage (G1 subchromatid exchanges or G2 whole chromatid exchanges) and that the majority of events occur in the first mitotic cycle.

In any case, it is possible to determine whether any detachments are produced in a two-strand stage at or after the third division, or in a four-strand stage at or after the second division. Second division two-strand events or first division four-strand events will yield half-male, half-female gynandromorphs. Later events



	TWO STRAND		FOUR STRAND			
	First Division	Second Division	First Division		Second Division	
			a	b	a	b
Sex Chromosome Constitution of First Four Cleavage Nuclei	X/y+Y	X/y+Y	X/y+Y	X/y+Y	X/y+Y	X/y+Y
	X/y+Y	X/y+Y	X/y+Y	X/y+Y	X/X̄Ȳ	X/X̄X̄
	X/y+Y	X/X̄Ȳ	X/X̄Ȳ	X/X̄X̄	X/X̄Ȳ	X/X̄Ȳ
	X/y+Y	X/X̄Ȳ	X/X̄Ȳ	X/X̄X̄	X/X̄Ȳ	X/X̄Ȳ

FIGURE 1.—A schematic representation of the detachment of generating event. The genotypes generated at two- and four-strand stages of the first or second embryonic mitosis are shown. Two different four-strand stage exchanges could occur. Events occurring at later divisions would yield mosaics with larger fractions of female tissue.

would yield gynandromorphs in which female tissue would predominate. Several tissues of a sample of detachment gynandromorphs were scored for sexual phenotype. Eyes were scored for shape (B^+ *vs.* $B/+$), forelegs were scored for sex combs, each side of the abdomen was scored for coloration, the external genitalia were scored for male or female structure and the wings were scored for size.

The results are shown in Table 8. Each structure is male about half the time, and the data fit expectations based on half-female, half-male mosaicism. Thus,

TABLE 8

Timing of the detachment event: gynandromorph phenotypes

Tissue	Number of gynandromorphs with male structures 0	1	2	Fraction male
Eyes	53	40	55	0.51
Forelegs	34	69	45	0.54
Wings	41	81	26	0.45
Left or right abdomen	46	45	57	0.54
Left or right genitalia	25	16	57	0.44
				Mean = 0.50

Gynandromorphs were classified as described in the text. Comparison with a 1:1 expectation of the observed numbers of flies with no male structures and flies with 2 male structures for a given landmark yields $\chi^2 = 8.55$ with 4 degrees of freedom; $p > 0.05$.

the gynandromorph-generating events occur no later than G1 of the second mitotic division, with most occurring earlier than that.

Rex-induced events could be homology-dependent, nonhomologous, or site-specific. Each of these possibilities predicts a different array of detachment products. Homologous exchange within duplicated, repeated-sequence regions would yield y^+Y products containing one set of the duplicated sequences or a variety of deficiencies and/or duplications. Nonhomologous exchange could also yield deficiencies or duplications, including aberrations extending beyond the duplicated segment. Among these would be sterile Y chromosomes. In addition, nonhomologous exchange could also yield y B ring-X chromosomes if exchange occurred on the other side of the centromere from that shown in Figure 1. Site-specific exchange would yield one or a few distinct detachment products.

Two observations make it likely that homology is involved. First, no ring-X chromosomes have been recovered. Though a small number of y B females were found among the several hundred thousand flies scored in the course of these experiments, progeny tests demonstrated that these females carried a linear, normal-sequence y B chromosome and a maternally derived X chromosome. These chromosomes could result from loss of the y^+ tip or from exchange between XY and X chromosomes, but they could not result from nonhomologous exchange on the Y^L side of the centromere. Their frequency is, in any case, so low that I am reluctant to ascribe their origin to *Rex*. Second, few if any exchanges are distal to the first Y-chromosome short-arm fertility factor, though that site is cytologically adjacent to the ribosomal sequences (J. KENNISON, personal communication). For example, in one sample of 200 detachment-bearing males, 172 (86%) were fertile. In a subsequent experiment, two males bearing each of these 172 Y chromosomes were retested. Though each male had a Y chromosome known to be fertile, 40 out of the 344 matings (12%) failed to yield offspring; a result not significantly different from that found for the detachment males themselves. Some males are infertile in any case, and the detachment-bearing males do not exhibit excess sterility. Thus, at most a small fraction, and likely none, of the detachment chromosomes are deficient for a fertility factor.

The bobbed constitution of the y^+Y chromosomes was examined to determine whether exchanges occur at many or at only a limited number of sites. *C(1)RM, y^2 su(w^a) w^a/y^+Y* × *y/y^+Y* stocks were established, starting with individual detachment-bearing males. Stock males were then crossed to *C(1)DX, y f bb$^-$/Y* females to expose the bobbed state of the y^+Y in daughters. Three phenotypes are associated with bobbed mutants: thinning and shortening of bristles, etching of the abdomen, and, in extreme cases, lethality. The visible phenotypes are quite variable from fly to fly and minor bristle abnormalities are obscured in this experiment by *f*. Therefore, penetrance, the fraction of females showing any of the bobbed stigmata, was used as a measure of visible bobbed phenotype. Decreased viability is reflected in reduced recovery of daughters. Two replicate matings were made for each of 172 y^+Y chromosomes and for 75 control chromosomes, with the results diagrammed in Figure 2. Wide variation in bobbed phenotype among y^+Y chromosomes is evident and is supported by the following statistical analysis.

The procedure is similar to an analysis of variance except that the data are in the form of numbers rather than measurements. Given two hypotheses, for example, one that a series of samples are all drawn from one population and another that each sample comes from a different population, the statistic:

$$G = 2 \times (\ln \max L_2 - \ln \max L_1) \ ,$$

where $\max L_1$ and $\max L_2$ are the maximum likelihoods under the two hypotheses, is distributed approximately as χ^2 with a number of degrees of freedom equal to the difference in number of parameters of the two models.

For the bobbed phenotype, the relevant hypotheses are that the detachment chromosomes are all drawn from one population or that each detachment is drawn from a different population. Under the first hypothesis the likelihood is:

$$L_1 = \prod_{i=1}^{N} p^{bb_i} (1-p)^{+_i} \ ,$$

where p is the probability of an individual fly expressing a bobbed phenotype, bb_i is the number of females bearing detachment i that are phenotypically bb, $+_i$ is the corresponding number of phenotypically + females, and N is the number of samples. L_1 is maximum when:

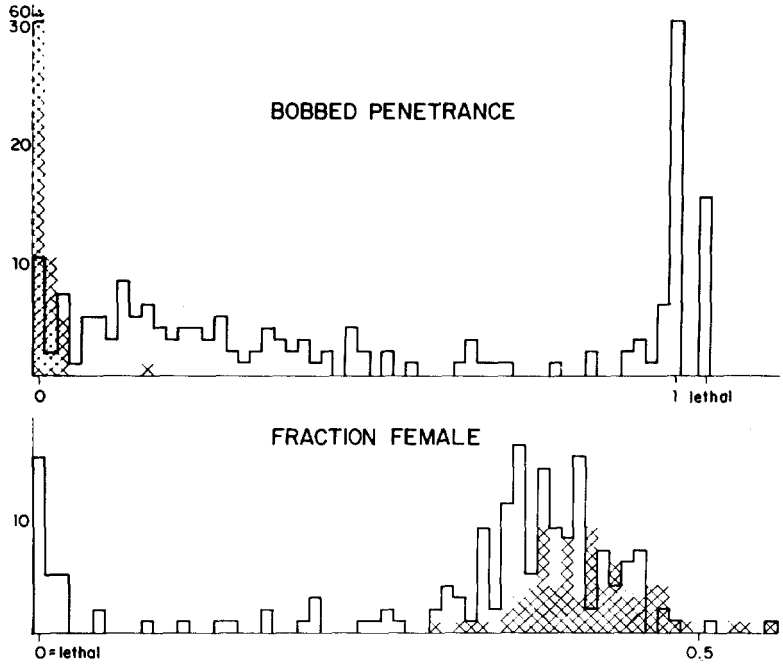
$$p = \frac{\Sigma bb_i}{\Sigma bb_i + \Sigma +_i} \ .$$

For the hypothesis that each detachment is distinct:

$$L_2 = \prod_{i=1}^{N} p_i^{bb_i} (1-p_i)^{+_i} \ ,$$

where p_i is the probability of a female bearing detachment i being bobbed. L_2 is maximum when:

$$p_i = \frac{bb_i}{bb_i + +_i} \ .$$



	BOBBED PENETRANCE	FRACTION FEMALE
Hypothesis	In Maximum Likelihood, No. of Parameters	
1) All samples from one population	-5234, 1	-15321, 1
2) Each detachment from a separate population	-1161, 157	-6087, 172
3) Each sample from a separate population	-246, 269	-2107 304*
Comparison of: 1 and 2 G=	8165, 156 d.f.	18468, 171 d.f.
2 and 3 G=	1830, 112 d.f.	7960, 132 d.f.

* For 40 detachments, only one of the pair of replicate tests was fertile.

FIGURE 2.—Bobbed phenotypes and viabilities of detachment products. One hundred seventy-two detachment y^+Y chromosomes were tested for bobbed phenotype in *C(1)DX*, y f bb^-/y^+Y females. Their y/Y male sibs provide a viability reference. Seventy-five normal Y chromosomes were examined in the same fashion. Results for detachment chromosomes are denoted by □, and those for controls by ×. The statistical design is discussed in the text.

Since some detachment chromosomes are bobbed lethal, the number of parameters is less than the 172 detachment chromosomes tested.

Were there no variation between replicates, the foregoing would reduce to the equivalent of a contingency test. However, *bb* expression is variable, and a third hypothesis must be considered: that each replicate is drawn from a different population. In that case:

$$L_3 = \prod_{i=1}^{N} p_{ia}^{bb_{ia}} (1-p_{ia})^{+_{ia}} p_{ib}^{bb_{ib}} (1-p_{ib})^{+_{ib}} ,$$

where p_{ia} and p_{ib} are the probabilities of a bobbed phenotype in the replicate tests of chromosome i. L_3 is maximum when:

$$p_{ia} = \frac{bb_{ia}}{bb_{ia} + +_{ia}} .$$

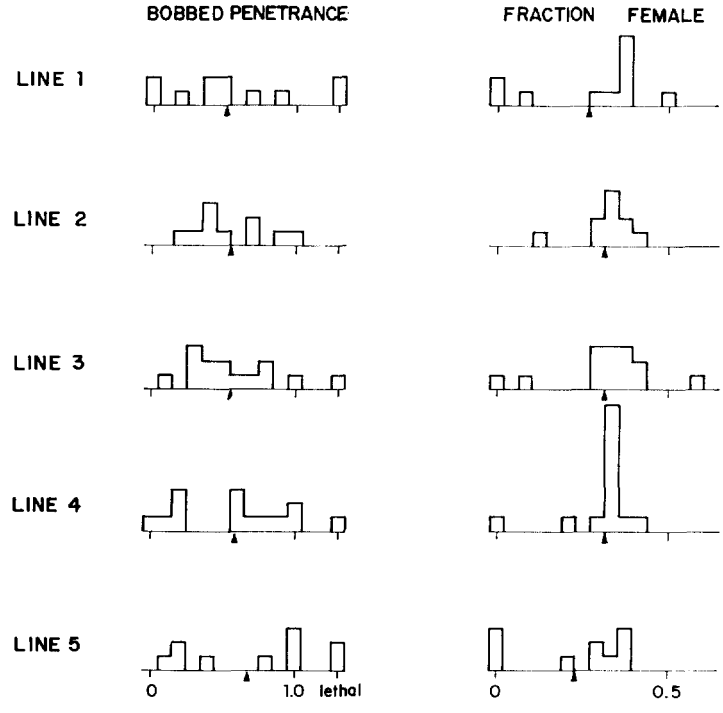
Since G is distributed approximately as χ^2, it is interpreted similarly. For the results shown in Figure 2, though there are significant differences in bobbed penetrance between individual sibships ($G = 1830$, 112 d.f.), the detachment chromosomes are clearly different from each other ($G = 8165$, 156 d.f.). Thus, most of the bobbed variation is ascribable to differences among detachment chromosomes, with a smaller residue of differences between replicate samples.

Analysis of viability variation differs only in the larger number of degrees of freedom since all 172 chromosomes are relevant to this test. Differences in viability are also mostly ascribable to differences among the detachment chromosomes. The distribution is bimodal, however, probably because only large deficiencies affect viability, given a background of massive numbers of maternally packaged ribosomes. A scatter plot of penetrance *versus* fraction female yields no apparent correlation of bobbed phenotype and viability, except for the most extreme *bb* chromosomes. A sample of these *Y* chromosomes was retested for *bb* phenotype and viability in $In(1)sc^{4L}sc^{8R}/y^{+}Y$ males, with concordant results (data not shown).

The detachment products clearly differ one from another with respect to ribosomal gene content. Before concluding that *Rex* causes exchanges at many points within the ribosomal complex, however, one must be certain that the variation is produced during the detachment event and did not pre-exist in the attached-*XY* stock. To test this, five stocks were established, starting with individual *XY* males. Males from each stock were crossed to y^2 *car Rex*/y females to generate a sample of detachments from each *XY* line, the $y^{+}Y$ chromosomes were stocked and individual $y/y^{+}Y$ males from each detachment stock were crossed to *C(1)DX* females. In this fluctuation test, variation among attached-*XY* chromosomes would yield differences among the five sets of detachments, while variation produced by the detachment event would yield differences within each set.

The results are shown in Figure 3. Statistical analysis follows a procedure analogous to that used for the preceding experiment. There is little variation among the five groups, compared to the large variation among detachments and, once again, there is a smaller, but significant, component of variation between replicates. Thus, the *bb* variation does not pre-exist, but is a consequence of the detachment event and the exchanges occur at multiple sites within the ribosomal complex.

Though the detachment chromosomes include a set of *bb* deficiencies, it is unlikely that they include many duplications. If a substantial number of duplications were produced, an excess of bb^{+} products would result. If pairing of duplicate regions overlapped randomly in either direction, at least half of the products would be bb^{+}. Fewer than 10% of the detachment chromosomes are bb^{+}; a num-



Hypothesis	ln of Maximum Likelihood,	No. of Parameters
1) All samples from same population	-2404,	1
2) Each attached-XY from a separate population	-2389,	5
3) Each detachment from a separate population	-690,	53
4) Each sample from a separate population	-626,	104

Comparison	G,	Degrees of Freedom
1 and 2	30,	4 d.f.
2 and 3	3399,	48 d.f.
3 and 4	127,	51 d.f.

Hypothesis	ln of Maximum Likelihood,	No. of Parameters
1) All samples from same population	-6970,	1
2) Each attached-XY from a separate population	-6948,	5
3) Each detachment from a separate population	-6541,	59
4) Each sample from a separate population	-6495,	118

Comparison	G,	Degrees of Freedom
1 and 2	43,	4 d.f.
2 and 3	814,	55 d.f.
3 and 4	91,	59 d.f.

FIGURE 3.—Sources of bobbed variation. Several $y+Y$ chromosomes derived from each of five *XY/O* lines were tested for bobbed phenotype and viability in $C(1)DX/y+Y$ males. The height of the shortest bar in each case corresponds to a test of one chromosome.

ber not very different from the 20% of cistrons that can be deleted without apparent phenotypic effect (K. TARTOF, personal communication). More frequent generation of deficiencies than duplications could result if the duplicated segments were of grossly unequal size, or if overlaps occurred most often in one direction. The bobbed region of the Y^S element of the XY is partially deficient, but whether that deficiency is sufficient to explain the absence of duplications must await cytological and molecular examination of the attached-XY chromosome and of the detachment products.

Despite these uncertainties, it is possible to construct a genetic map of the segment within which exchanges occur (whatever its actual physical structure). Since each detachment is an independent event, the fraction of all detachments with a phenotype less severe than a given phenotype represents the fraction of exchanges occurring to one side of a genetically defined point. Thus, if the y^+Y chromosomes are ranked according to phenotype, a map may be drawn associating phenotype with the extent of deletion of the region within which exchanges occur. The relative distance generated is analogous to a mitotic map distance based on selection of a distal marker. An example of this, derived from the data used to generate Figure 2, is shown in Figure 4. Except at the extremes, where there are enough copies for normal phenotype or where all females are *bb* or lethal, the relationship between bobbed penetrance and genetic length is a smooth curve suggesting that the exchanges occur at random in the interval.

DISCUSSION

The foregoing experiments suggest that *Rex* is a maternally acting, dominant inducer of mitotic exchange within the ribosomal cistrons of the attached-*XY*

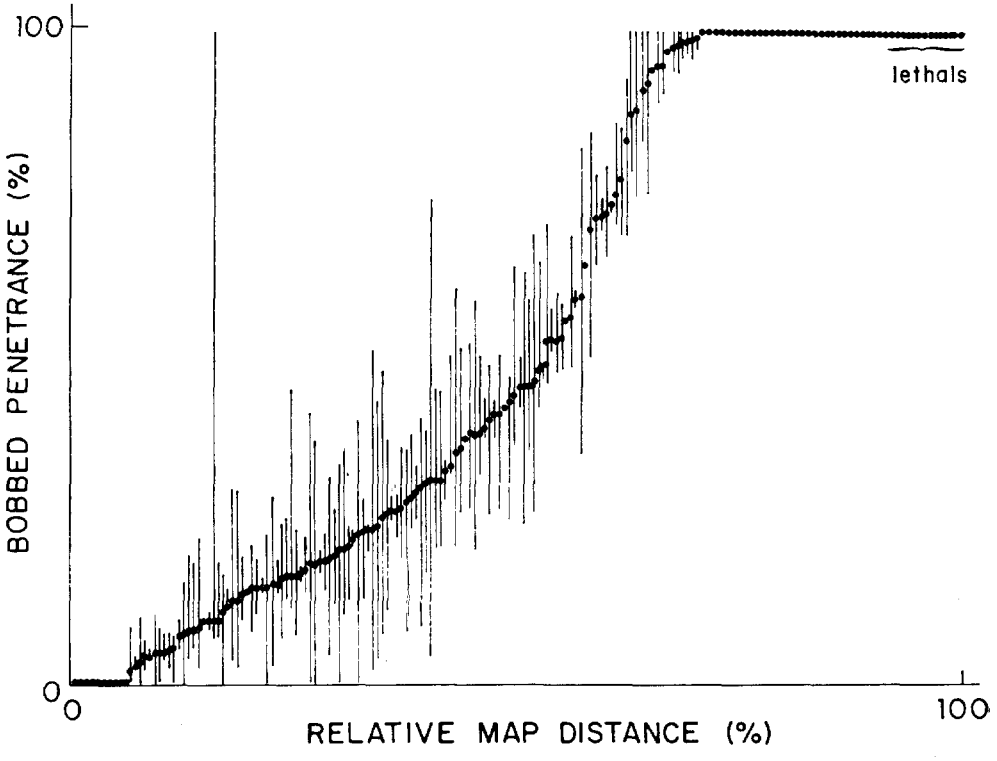


FIGURE 4.—Genetic map of the region of detachment generating exchanges. The detachment chromosomes diagrammed in Figure 2 were ranked according to bobbed phenotype. The relative map distance of a given exchange from the exchange site closest to the centromere is the fraction of detachments having a weaker phenotype. The dashed lines indicate the values observed for the replicate tests of each detachment chromosome.

chromosome. Though its effect is unconventional, *Rex* itself behaves in an entirely Mendelian fashion. Whether the apparent restriction of exchanges to the ribosomal DNA is a consequence of some specificity of *Rex*, or of the particular structure of the attached-*XY* used, is not yet known. It is also possible that pairing configurations other than the one yielding detached *Y* chromosomes could occur.

Other genetic elements have been described that are either mitotically unstable or induce mitotic chromosome misbehavior, but these instances have usually been detected by loss of chromosomes and therefore do not yield a product from which inferences about mechanism can readily be made. Examples include ring-*X* chromosome loss (reviewed by HALL, GELBART and KANKEL 1976; LEIGH 1976), *pal* (BAKER 1975), *mit* (GELBART 1974) and claret (*ca* in *D. simulans* STURTEVANT 1929; ca^{nd} in *D. melanogaster* DAVIS 1969). Though many uncertainties remain in the analysis of *Rex*, the results to date raise some interesting possibilities:

(1) A maternally determined system exists that is important to proper chromosome behavior in early embryos even prior to fusion of male and female pronuclei (SONNENBLICK 1950; DÄVRING and SUNNER 1973).

(2) Whole chromatid exchanges at mitotic G1 are produced by a defect in this system.

(3) This error can result in homology-dependent exchange between duplicated ribosomal gene complexes, and may be related to normal processes of maintenance of ribosomal gene copy number (TARTOF 1975, RITOSSA 1976).

(4) A genetic map of a ribosomal cluster can be generated. Though its physical meaning is obscured by uncertainties about the structures of the attached *XY* and the detachment products, the lack of clustering hints at a reasonably direct relationship between copy number and penetrance and at a broad distribution of exchange sites.

(5) A set of ribosomal complex deficiencies is now available where the remaining cistrons probably have common endpoints. However, whether these copies are *X*-chromosome derived, or *Y*-chromosome derived, or a mixture, is not yet known.

(6) The chromosome in which *Rex* was found contained a deficiency [$Df(1)w^{rJ1}$] that is itself a product of unequal recombination. The event that yields $Df(1)w^{rJ1}$ and some other deficiencies in the same region is recurrent in some crosses, and never occurs in others (JUDD 1961). It is possible that *Rex* is responsible for the differences between genotypes in which such unequal exchanges do and do not occur. It is possible to test this, and, if it proves to be true, a system would be identified that affects generalized illegitimate exchange important for maintenance not only of repeated gene families like the ribosomal complex, but of other duplicated segments as well. Such a system could be of significant evolutionary interest.

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