NON-MENDELIAN FEMALE STERILITY IN *DROSOPHILA MELANOGASTER*: HEREDITARY TRANSMISSION OF *I* FACTOR

G. PICARD

Laboratoire de Génétique, Université de Clermont-Ferrand, B.P.45, 63170 Aubiere, France

Manuscript received August 11, 1975
Revised copy received December 24, 1975

ABSTRACT

Systematic crosses between various strains of *Drosophila melanogaster* lead in some cases to partly sterile F_1 females. Two main classes of strains, inducer and reactive, may be recognized on the basis of the fertility of F_1 female progeny. Females which may show incomplete sterility (*SF* ♀) arise only when reactive females are crossed with inducer males, other crosses, including the reciprocal, producing only fertile F_1 females.

SF sterility appears as the result of an interaction between two factors, *R* brought into the initial cross by the reactive mother and maternally inherited, and *I* brought by the inducer father. The present paper reports on the hereditary transmission of *I* factor. It is shown that when transmitted through heterozygous males, bearing chromosomes of both inducer and reactive origin, *I* factor may be strictly linked to any one of the three major chromosomes of inducer strains. Such chromosomes carrying *I* factor were called inducer chromosomes. When transmitted through heterozygous females, this Mendelian behavior fails to hold, and non-inducer chromosomes coming from reactive strains may become inducer independently of the production of recombined gametes. This phenomenon was called chromosomal contamination. This contamination occurs even between nonhomologous chromosomes.

IT was previously reported (PICARD 1971) that crosses between different strains of *Drosophila melanogaster* lead in some cases to partly sterile F_1 females. On the basis of the fertility of F_1 females, strains can be distributed into two main classes, inducer and reactive (PICARD *et al.* 1972a). A third class, called neutral, was also distinguished, but at present, a single strain has been found to belong to this class. Crosses between strains within the same class, and all crosses involving the neutral strain breed fertile F_1 daughters. Females showing incomplete sterility may arise only when females from a reactive strain are crossed with inducer males. F_1 females resulting from such crosses are denoted *SF* females (BUCHETON 1973). Crosses between inducer females and reactive males lead on the contrary to normally fertile daughters. These F_1 females produced by a mating the reciprocal of which produces *SF* females are denoted *RSF* females.

SF sterility results from the failure of some eggs to complete embryonic development, those hatching successfully giving flies which do not exhibit any conspicuous aberration. According to the choice of the two reactive and inducer par-

ental strains which are crossed, the hatching percentage of the eggs laid by the *SF* females is very variable, and may take any possible value, from zero upwards. Thus, the neutral strain may be viewed as an extreme type of either of the two other classes, unable to give any noticeable sterility.

SF sterility may be distinguished without ambiguity from other kinds of genetically determined sterility owing to three characteristic features:

1) The hatching percentage of the eggs rises regularly when *SF* females age (PICARD 1971).

2) Hatchability rises quickly but reversibly when egg-laying *SF* females are put at a temperature of 30° (PICARD 1971).

3) The probability of death of embryos does not depend on their own genotypes, and therefore is quite independent of the genetic origin of the mates of *SF* females (paper in preparation).

A survey of more than 100 strains has shown that all the strains coming from flies caught in the wild during the last few years are inducer while the strains coming from various European laboratories are distributed into the three classes. Therefore, the inducer condition appears as the normal condition for *D. melanogaster*, while the reactive condition is the outcome of some genetic change which has taken place in some laboratory isolates.

For the convenience of genetic analysis, *SF* sterility may be viewed as the result of an interaction between two factors, *R* brought by the reactive mother, and *I* brought by the inducer father (PICARD *et al.* 1972b).

The way in which *R* factor is inherited is still under investigation, but the first results seem to indicate that it may be a population of cytoplasmic particles maternally inherited (BUCHETON 1973; BUCHETON et PICARD 1975). *R* factor exhibits a large variability and generally a reactive strain is a mixture of *strong* reactive females the cross of which by inducer males leads to highly sterile *SF* females and *weak* reactive females, which in the same conditions lead to *SF* females showing nearly normal fertility. All the intermediate types also exist. *Strong* or *weak* reactive families which include only *strong* or *weak* reactive females can be obtained from original strains either by selection or by spontaneous drift (PICARD *et al.* 1972a).

The present paper reports the results of experiments bearing on the hereditary transmission of *I* factor. They provide evidence for a rather unusual genetic behavior. When heterozygous males carrying chromosomes originating from inducer and reactive strains together are mated with reactive females, they yield *SF* females only among their daughters receiving chromosomes of inducer origin. This strict linkage with chromosomes can be observed through several successive generations of heterozygous males. However, this Mendelian transmission operates only in the male germ line. Indeed, in *SF* and perhaps in *RSF* females, chromosomes of reactive origin undergo, often with a high frequency, an apparently irreversible genetic change and become inducer. This change takes place independently of the production of recombined gametes. The phenomenon has been called chromosomal contamination.

MATERIALS AND METHODS

Genetic symbols are those used by LINDSLEY and GRELL (1967). Flies were grown on the axenic food described by DAVID (1969), at the temperature of 20° which seems to be the optimal temperature for having *SF* fertility as low as possible.

Stocks of Drosophila melanogaster used in the experiments

a) *reactive*

Two stocks used in this paper are *strong* reactive stocks which were established by selection, following the method described in PICARD *et al.* (1972a), dating from some original reactive strains maintained in our laboratory. They were selected for breeding highly sterile *SF* females in the crosses with inducer males of the Luminy stock. These two strains have been denoted:
1) *se* $F_{\bar{5}}$, which comes from an original strain homozygous for the sepia mutation.
2) LH_{12}, which comes from an original strain (*LH*), the genotype of which is $In(1)sc^{S1L}sc^{sR+S}$, $sc^{S1}sc^{s}w^{a}B/In(1)sc^{S1L}sc^{sR+S}$, $sc^{S1}sc^{s}w^{a}B$; *In(2L+2R)Cy/Pm*; *H/Sb*. The inversions carried by the *X* chromosomes will be indicated by *M-5* (= *Muller 5* = *Basc*) in all the Figures and Tables. The *In(2L+2R)Cy* chromosome bears the inversions *In(2L)Cy* and *In(2R)Cy*. These two chromosomes will be used as inhibitors of crossing over. In contrast, the chromosome *2* bearing *Pm* (= bw^{V1}) and the chromosomes *3* bearing *H* and *Sb* does not inhibit the production of recombined gametes.

Three other stocks were also used, without any preliminary selection:

1) *w/w*; *In(2L+2R)Cy/Pm*; *H/Sb* obtained by crosses between the above mentioned original *LH* strain and a reactive strain homozygous for the mutation *white*, maintained in our laboratory.

2) $In(1)sc^{S1L}sc^{sR+S}$, $sc^{S1}sc^{s}w^{a}B/In(1)sc^{S1L}sc^{sR+S}$, $sc^{S1}sc^{s}w^{a}B$; +/+; +/+ comes from the John Innes Institute, Norwich (England) and was kindly supplied by DR. B. J. HARRISON. (Stock No. 26 in *Dros. Inf. Serv.* **48**)

3) +/+; +/+; *In(3LR)DcxF/Sb* comes from the Dept. of Genetics of Umeå University (Sweden) and was kindly supplied by PROF. B. RASMUSON. (Stock No. 3017 in *Dros. Inf. Serv.* **48**).

b) *Inducer*

1) $In(1)sc^{S1L}sc^{sR+S}$, $sc^{S1}sc^{s}w^{a}B/In(1)sc^{S1L}sc^{sR+S}$, $sc^{S1}sc^{s}w^{a}B$; +/+; +/+ comes from the Dept. of Genetics of Birmingham University (England) and was kindly supplied by PROF. J. L. JINKS. (Stock No. 25 in *Dros. Inf. Serv.* **46**).

2) +/+; *In(2L+2R)Cy/Pm*; *H/Sb* comes from the Laboratory of Genetics of Lyon University (France), and was kindly supplied by PROF. J. DAVID. In this stock, only the *In(2L+2R)Cy* chromosome which bears the inversions *In(2L)Cy* and *In(2R)Cy* was used as crossing-over inhibitor.

3) Luminy Wild-type strain coming from flies caught in the wild in 1969 in Southern France and then maintained in our laboratory.

All the stocks used in this work carry a wild chromosome *4*.

Measurement of the hatching percentages of the eggs

Two or three days after emergence, mated females were individually put in a culture vial containing food stained with some carbon black. A sample of about 50 eggs was collected on this food during about 48 hours. The eggs, which were easily seen on the black background were recorded as hatched or not hatched 48 hours later. Females were mated with their brothers since it has been shown that the hatching percentage of the eggs did not depend on the males used to fertilize *SF* females.

RESULTS

Linkage of I *factor to inducer strains chromosomes in spermatogenesis*

I factor transmission in spermatogenesis was studied by mass crosses involving in each case about 15 flies of each sex. The two reciprocal crosses between the

Luminy +—(*i*)/+—(*i*); +—(*i*)/+—(*i*); +—(*i*)/+—(*i*)* inducer stock and the *In(1)sc*^{S1L}*sc*$^{S8R+S}$, *sc*^{S1}*sc*^{S}*w*^{a}*B* —(*r*); *In(2L+2R)*Cy —(*r*)/*Pm* —(*r*); *H* —(*r*)/*Sb* —(*r*) reactive stock (LH_{12}) yield respectively +—(*i*); Cy—(*r*)/+—(*i*); *Sb*—(*r*)/+—(*i*) and *In(1)* . . . —(*r*); Cy—(*r*)/+—(*i*); *Sb*—(*r*)/+—(*i*) heterozygous males which are mated with *se* F_5 *females*. According to the gametes they have received from their fathers, and disregarding chromosome 4, the daughter progeny of each cross can be distributed into four genotypic classes. The fertility of about 50 individual females of each class is measured, as described in MATERIALS AND METHODS. Coming from reactive mothers, females will be putitively sterile when they have received *I* factor from their fathers.

Using the *se* F_5 reactive stock instead of the Luminy stock, these matings were repeated as control experiment.

The four histograms of Figure 1 give the results observed with females bearing either none of the three Luminy major chromosomes (a) or only one of them (b, c, d). It can be seen that in the first genotypic class (a), female fertility does not fall significantly below control values, while in the three latter classes, some (c) or all (b, d) females show a high degree of sterility. Data concerning the females which bear several Luminy chromosomes will not be presented here in detail. All of them display a very high sterility, the hatching percentages of the eggs being zero for most of them and rising in no case above 3%.

Therefore, through spermatogenesis every one of the three Luminy major chromosomes appears liable to carry what has been called the *I* factor. However, the chromosome *4* seems unable to do so, since all females of the first genotypic class (Figure 1a) show a normal fertility although statistically half of them possess a chromosome *4* originating from the Luminy strain. Another point concerns females bearing only the chromosome *2* coming from the Luminy strain (Figure 1c). It is clear that they belong to two well-defined classes: some of them show a high level of sterility whereas the others are as fertile as control females. This heterogeneity may reflect a real polymorphism, if two types of chromosomes *2* coexist in the Luminy stock, only one of them bearing *I* factor. This point is under investigation and the first results seem to indicate that a polymorphism exists.

On the basis of the ability to carry *I* factor in spermatogenesis, we can therefore define two types of Luminy chromosomes: the inducer (*X*, *3* and some *2*) and the non-inducer (*4* and the other 2). These last chromosomes do not appear to behave in a different way from the chromosomes of the reactive strains. These chromosomes will be denoted in this paper i^+ for inducer chromosomes, i^o for non-inducer chromosomes originating from inducer strains and *r* for chromosomes originating from reactive strains.

Stability of I *factor linkage to* i^+ *chromosomes through successive generations of males*

An experiment was carried out to ascertain the stability of *I* factor linkage to i^+ chromosomes when they are transmitted through several successive genera-

* Chromosomes originating from an inducer or a reactive strain are respectively indicated by the indexes -(*i*) or -(*r*).

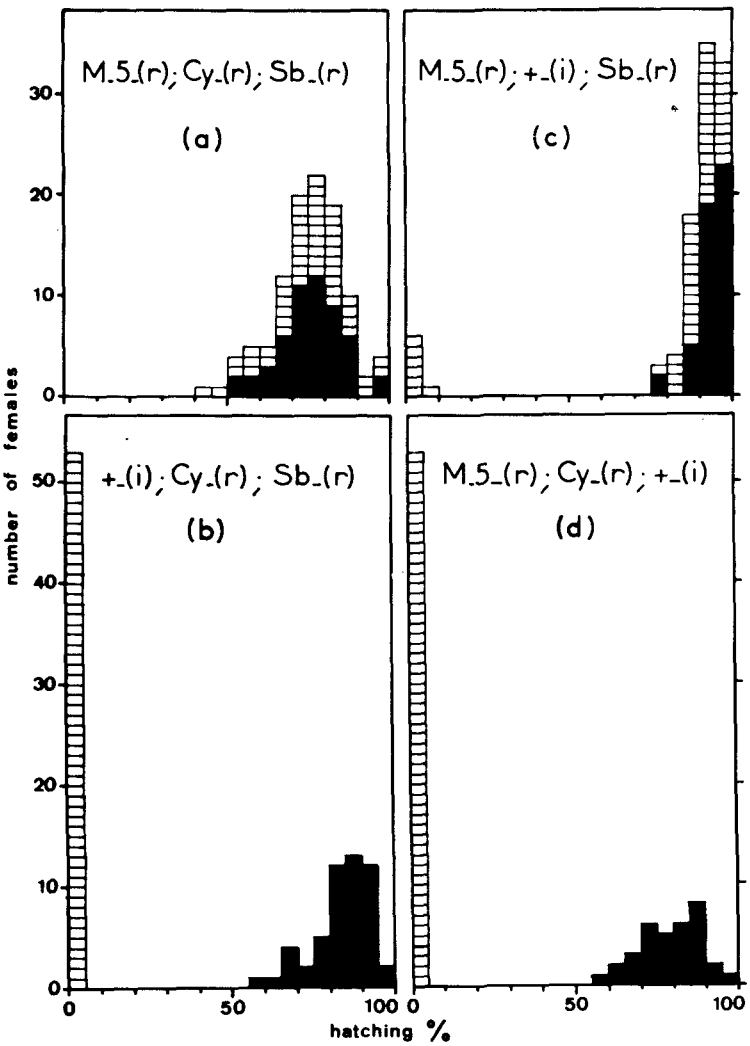

FIGURE 1.—Linkage of *I* factor to inducer strains of chromosomes in spermatogenesis. The histograms show the fertility of females carrying the indicated paternal genomes. Each female is figured by a white square. Control females, bearing *se* F_5 chromosomes instead of the *+—(i) Luminy* chromosomes are figured by black squares.

M—5—(r) = *In(1)sc*^{S1L}*sc*$^{S1R+S}$, *sc*^{S1}*sc*^{S}*u*^{a}*B—(r)*

Cy—(r) = *In(2L+2R)Cy—(r)*

tions of heterozygous i^+/r males in the presence of *R* factor. Thirty heterozygous *males +—(r); Cy—(i)/+—(r); Sb—(i)/+—(r)* bred from a mass cross between 20 *+—(r)/+—(r); +—(r)/+—(r); +—(r)/+—(r)* reactive *se* F_5 females and 20 *+—(i); In(2L+2R)Cy—(i)/Pm—(i); H—(i)/Sb—(i)* inducer males, were backcrossed to 20 *se* F_5 females. This same backcross was then repeated during ten generations, and at each one of them, the fertility of 40 individual females

bearing the marker *Cy—(i)* and 40 individual females bearing the marker *Sb—(i)* was measured. Simultaneously, a similar experiment was performed with heterozygous males *+—(r);Cy—(i)/+—(r);H—(i)/+—(r)*, and at each generation of backcross, the fertility of about 40 individual females bearing the marker *H—(i)* was measured. In the progeny of the first generation of backcross, the fertility of 86 females not bearing any of the chromosomes *Cy—(i)*, *Sb—(i)* or *H—(i)* was also measured. These females show a fertility which is very similar to that of females of the *se* F_5 stock. The average value of the individual hatching percentages is 89.8%, with a standard deviation of 10.2. In contrast, females from the same progeny, but bearing any one of these chromosomes exhibit complete sterility (Table 1, second generation). These results establish the inducer character of the three chromosomes *Cy—(i)*, *Sb—(i)*, *H—(i)*, and the non-inducer character of the chromosome *4* of the inducer stock, since it was carried by half of the 86 fertile females tested. Results observed during the successive generations are given in Table 1. The great majority of females bearing any of the three inducer chromosomes show complete sterility, and the average values of the hatching percentages are always very low (below 1%). When they are above zero this means that some females (four at most), in the sample of about 40, laid some eggs which hatched successfully. The hatching percentage values of these individual females are always below 20%.

In spite of such minor variations, all the females of the indicated genotypes therefore show a high degree of sterility, and even in the last generation, no normally fertile female was ever found. It may be concluded that inducer chromosomes keep their ability to carry *I* factor through several successive generations of heterozygous males.

Thus, *I* factor appears as strictly linked to chromosomes i^+ and behave like genes distributed on these chromosomes. Preliminary assays to map *I* factor have given rather curious results since almost all the chromosomes yielded by $SF(i^+/r)$ females were found inducer. To investigate this point, it was first necessary to look at the stability of the non-inducer character of the *r* chromosomes when they are transmitted heterozygously. This stability was studied first

TABLE 1

Stability of the inducer character of the Cy-(i), Sb-(i) *and* H-(i) *chromosomes through successive generations of males*

Genotypes of ♀ *(paternal genomes)	Generations 2	3	4	5	6	7	10	11
†+-(r);Cy-(i);+-(r)	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00
+-(r);+-(r);Sb-(i)	0.00	0.00	0.04	0.00	0.95	0.00	0.64	0.48
+-(r);+-(r);H-(i)	0.00	0.00	0.23	0.00	0.30	0.14	0.44	0.38

* Since all the females come from *se* F_5 mothers, their genotypes differ only by the genomes they have inherited from their fathers.

† The +-(r) chromosomes come from the reactive stock *se* F_5. Since the gene sepia is never used as a genetic marker along this work, it was not indicated in the genotypes.

Each number is a percentage which is the average of about 40 hatching percentage measurements.

in heterozygous males, and then in heterozygous females in which the production of recombined gametes was reduced by the use of inversions.

Stability of the non inducer character of the r *chromosomes through successive generations of males*

The previous experiments show that *r* chromosomes exhibit a non-inducer character when they are transmitted heterozygously in males originating from reactive mothers, since females which do not bear any of the chromosomes from

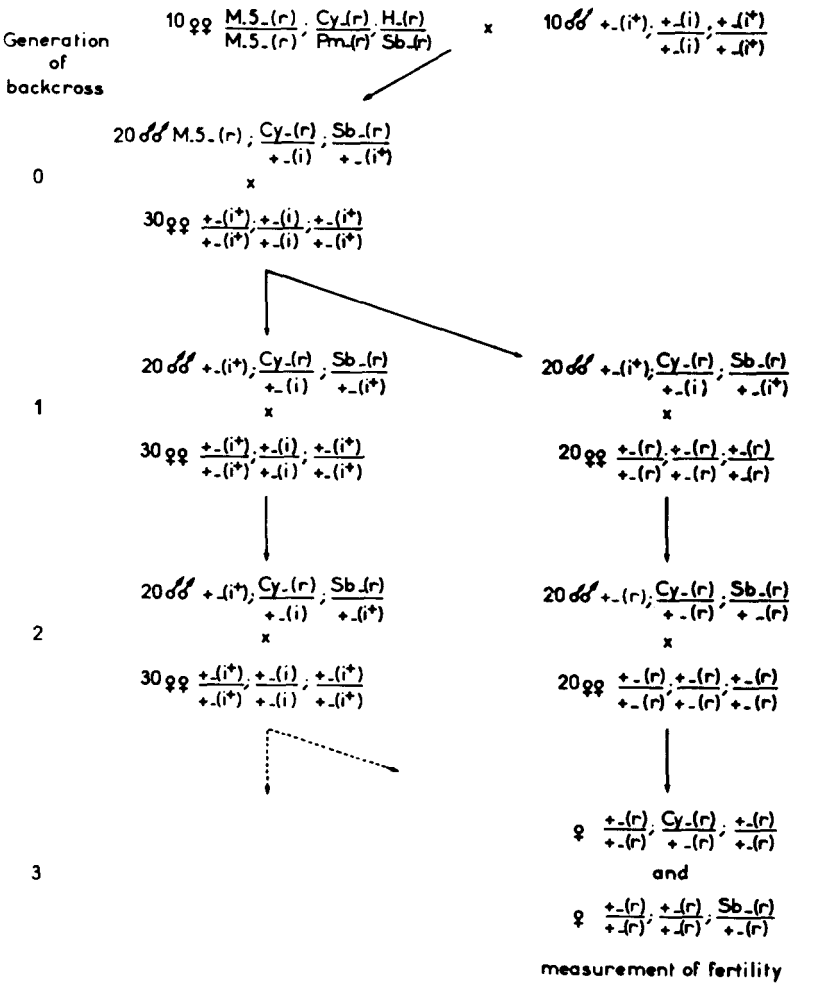

FIGURE 2.—Stability of the non-inducer character of the *Cy-(r)* and *Sb-(r)* chromosome through successive generations of males. *M-5-(r)* = *In(1)sc^{s1L}sc^{s8R}+^{S},sc^{s1}sc^{s8} w^{a} B-(r)*; *Cy-(r)* = *In(2L+2R)Cy-(r)*

The *+-(i+)* chromosomes are inducer chromosomes coming from the Luminy stock. However, since the chromosomes *2* of this stock may be either *i+* or *i^{o}*, they were indicated *-(i)* in this Figure.

The *+-(r)* chromosomes come from the reactive stock *se* F_5.

inducer origin are normally fertile. The present experiment was carried out to show whether the *r* chromosomes keep their non-inducer character, even when they are transmitted heterozygously with i^+ chromosomes during several successive generations, in males coming from inducer mothers. The experimental scheme is indicated on Figure 2.

Heterozygous males bearing the *Cy—(r)* and *Sb—(r)* chromosomes coming from the *In(1)*$sc^{S1L}sc^{s8R+S}$, $sc^{S1}sc^{s8}$ w^aB*—(r)*; *In(2L+2R)Cy—(r)/ Pm—(r)*; *H—(r)/Sb—(r)* reactive stock (LH_{12}) were backcrossed during 11 successive generations to Luminy females. Every two generations, *Cy—(r)* and *Sb—(r)* chromosomes were tested for an eventual ability to carry *I* factor. For this purpose, two successive backcrosses to *se*F_5 reactive females are necessary to eliminate all the i^+ chromosomes and to obtain females which bear a single marked chromosome, either *Cy—(r)* or *Sb—(r)*. The fertility of about 40 individual females belonging to each of these two genotypic classes was then measured. Since the Luminy chromosome *4* is known as non-inducer, the finding of some sterile females will indicate that some *Cy—(r)* or *Sb—(r)* chromosomes have become inducer. As a control experiment involving *r* chromosomes which have never been associated with i^+ chromosomes, *In(1)*$sc^{S1L}sc^{s8R+S}$, sc^{S1} $sc^{s}w^aB$*—(r)*; *In(2L+2R)Cy—(r) / Pm—(r)*; *H—(r) / Sb—(r)* reactive males were crossed with *se*F_5 females, and heterozygous sons carrying the *Cy—(r)* and *Sb—(r)* chromosomes were backcrossed to the same females. The fertility of about 40 daughters bearing the *Cy—(r)* chromosome and 40 daughters bearing the *Sb—(r)* chromosome was measured.

The histograms of Figure 3 show that the fertility of females bearing the *Cy—(r)* or the *Sb—(r)* chromosomes coming either from the first or from the eleventh generation of backcrosses is quite similar to that of control females. The same is true for females of the same genotypes found in the intermediate generations, and the data concerning them will not be presented here.

Therefore, passing through successive generations of heterogygous males did not change the character of the chromosomes *Cy—(r)* and *Sb—(r)* from reactive origin. Similarly, in the previous experiment, it was demonstrated that in the same circumstances, chromosomes from inducer origin keep their original character. Therefore, as long as chromosomes are maintained in males, what has been called *I* factor exhibits an orthodox Mendelian behavior.

Stability of the non-inducer character of r *chromosomes through heterozygous females*

The chromosomes from reactive origin of *SF* or *RSF* females can be recovered in their male offspring and assayed as in the previous experiments, for their possible ability to carry *I* factor. The sterility of *SF* females does not prevent the recovering of their chromosomes since it was shown (PICARD 1971) that the hatching percentage of the eggs rises with aging of these females. To inhibit genetic recombination some chromosomes bearing dominant markers, linked to inversion complexes, were used.

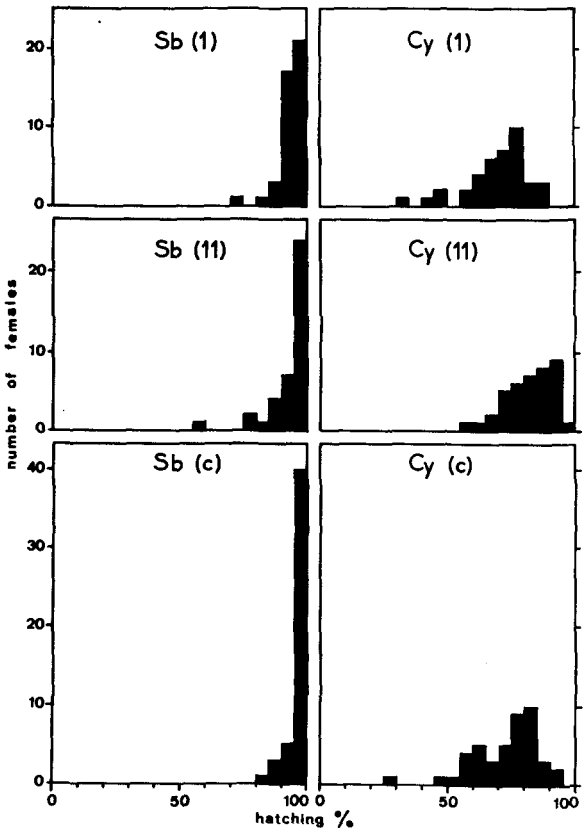

FIGURE 3.—The histograms show the fertility of females carrying either a *Sb-(r)* or an *In(2L+2R)Cy-(r)* chromosome coming from their father. On the top and the middle rows, the females have a reactive mother, and the chromosomes tested for their ability to induce *SF* sterility have been carried in heterozygous males through respectively 1 and 11 generations of backcrosses. Histograms of the lower row (c) pertain to control females of the same genotype which are entirely of reactive origin (see text).

Two sets of experiments were performed. In the first, *SF* or *RSF* females with a whole paternal genome from inducer origin were used. In the second set, designed to test the possibility of an interaction between nonhomologous chromosomes, the paternal genomes of *SF* females included only one or two chromosomes from induced origin.

Within the first set, four experiments were performed with the same general scheme (Figure 4). They differ only in the first initial cross which gives rise to the G_1 heterozygous females.

In the first experiment (a), *SF* G_1 females were obtained in which recombination was inhibited on the first and on the second chromosomes, but not on the third. The average value of the hatching percentage of the eggs laid by a set of these *SF* females is 10.7% ± 1.0. Their sons bearing the *w—(r)* and *Cy—(r)* chromosomes from reactive origin, and a possibly recombined third *Sb* chromo-

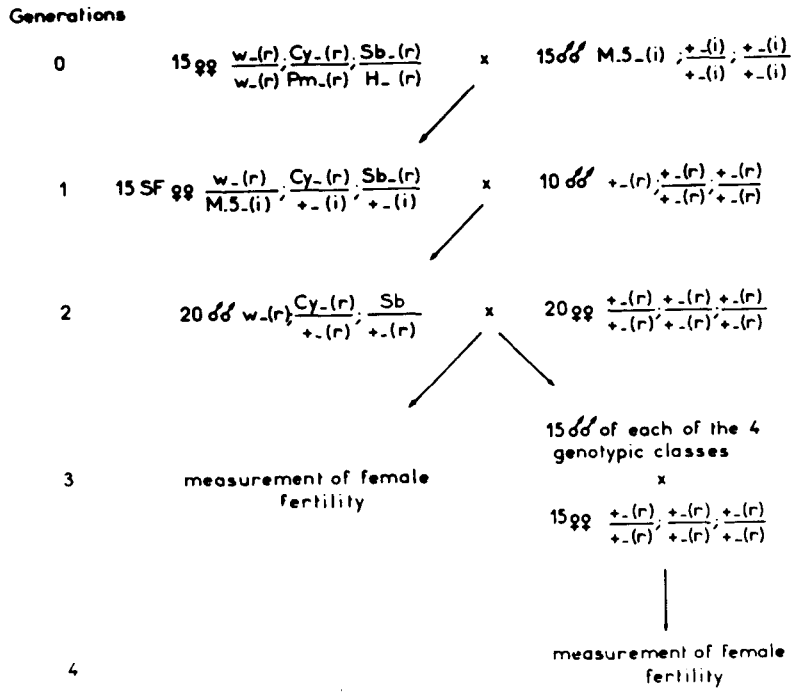

FIGURE 4.—Scheme of experiment (a)
M-5-(i) = *In(1) sc^{S1L}sc^{8R+S}, sc^{S1}sc^{8} w^{a} B-(i)*
Cy-(r) = *In(2L+2R)Cy-(r)*
+-(i) are chromosomes coming from the inducer stock *In(1) sc^{S1L}sc^{8R+S}, sc^{S1}sc^{8} w^{a} B-(i)/In(1) sc^{S1L}sc^{8R+S}, sc^{1}sc^{8} w^{a} B-(i)*;*+-(i)/+-(i)*; *+-(i)/+-(i)*
+-(r) are chromosomes coming from the *se* F_5 stock.

some, were crossed with *se* F_5 reactive females. The fertility of about 50 G_3 daughters belonging to each of the four genotypic classes was measured. Their brothers of the four possible genotypes were mated with *se* F_5 reactive females, and the fertility of about 40 G_4 daughters of each genotypic class was also measured.

In the second experiment (b), the reciprocal cross, *In(1)sc^{S1L}sc^{8R+S},-sc^{S1}sc^{8}w^{a}B—(i)/In(1) sc^{S1L}sc^{8R+S},sc^{S1}sc^{8}w^{a}B—(i);+—(i)/+—(i);+—(i)/—+(i)* inducer females by *w(—r);In(2L+2R)Cy—(r)/Pm—(r);H—(r)/Sb—(r)* reactive males was used to obtain the G_1 females. Therefore although their genotype was the same as in the first experiment these flies were fertile *RSF* females.

The two last experiments (Ca) and (Cb) were control experiments designed to test the fertility of females of the various genotypes without any involvement of the *I* factor. This was achieved quite simply by using in the initial cross the *In(1)sc^{S1L}sc^{8R+S},sc^{S1}sc^{8}w^{a}B—(r)/In(1)sc^{S1L}sc^{8R+S},sc^{S1}sc^{8}w^{a}B—(r);+—(r)/+—(r); +—(r)/+—(r)* reactive stock as a substitute for the inducer stock of the same genotype used in the experiments (a) and (b).

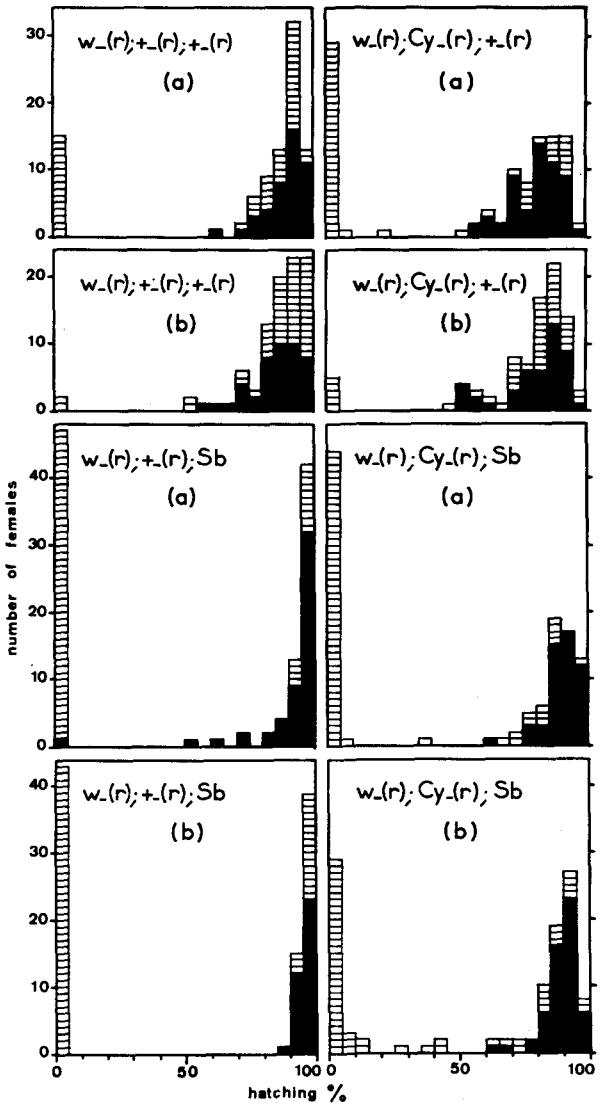

FIGURE 5.—The histograms show the fertility of G_3 females bearing the *w-(r)* and *In(2L+2R) Cy-(r)* reactive originating chromosomes and the *Sb* eventually recombined chromosomes inherited from the heterozygous G_1 *SF* (a) or *RSF* (b) females. The genotypes of the females are only different by their paternal genomes which were indicated in each histogram, while the maternal genomes, coming from *se* F_5 females are always +-*(r)*; +-*(r)*; +-*(r)*. Each white square represents the fertility of one G_3 female coming from the experiments (a) or (b). Females coming from the control experiment Ca or Cb are indicated by black squares.

Figure 5 and Table 2 show respectively the results of the fertility measurements of G_3 and G_4 females. The Table indicates the number of sterile females of the total number of tested G_4 females for each genotypic class obtained in the four experiments. As for G_3 females, (Figure 5) the distinction between sterile

and fertile females is very easy. These results give evidence for the three following points:

1) Although they do not carry any chromosome from inducer origin (except chromosome *4* which is statistically present in one fourth of them) some G_3 females of the experiments (a) and (b) (Figure 5) exhibit a high degree of sterility with regard to control females obtained in the experiments (Ca) and (Cb). These sterile females must have therefore inherited *I* factor from their father.

2) Table 2 indicates that in the generation G_4 *I* factor appears to be linked to the *Cy—(r)* and *Sb* chromosomes. Indeed, in experiments (a) and (b), sterile females are found almost uniquely in the genotypic classes bearing either one of these two chromosomes, or both of them; while among the 481 G_4 females which do not bear any of them only 2 show some sterility. Such exceptional females may carry an inducer chromosome *4* originating from the *In(1)* $sc^{s1L}sc^{s R+s}$, $sc^{s1}sc^{s}w^{a}B—(i)/In(1)sc^{s1L}sc^{s R+s}$,$sc^{s1}sc^{s}w^{a}B—(i)$; $+—(i)/+—(i)$; $+—(i)/+—(i)$ inducer stock, or may exhibit a kind of sterility which bears no relation to the *SF* phenomenon. Whatever the case, even if some chromosomes *4* are inducer, this can account only for a very small part of the female sterility found in experiments (a) and (b). It can be concluded therefore, that in the *SF* as well as in the *RSF* G_1 females, some of the chromosomes *w—(r)*, *Cy—(r)* and *Sb—(r)* of reactive origin and associated heterozygously with i^+ chromosomes became inducer and acquired the ability to carry *I* factor through successive males.

3) In the *SF* and *RSF* females, recombination is free only on the chromosome *3*. Consequently, the *Sb* chromosomes tested in the G_3 and G_4 females for their ability to carry *I* factor may be recombined chromosomes which can bear any segments of the $3i^+$ chromosomes of the inducer stock. On the contrary, the *w—(r)* and *In(2L+2R)Cy—(r)* chromosomes may be assumed entirely from reactive origin, except for some residual recombinations which may probably occur in spite of the use of inversions. Among the G_3 females bearing the *Sb*

TABLE 2

Linkage of I *factor to the* Cy-(r) *and* Sb *chromosomes carried by* G_3 *males*

	Genotypes of the G_3 ♂♂ crossed with *se* F_5 ♀♀								
	$+\text{-}(r)\frac{Cy\text{-}(r)}{+\text{-}(r)}$	$\frac{+\text{-}(r)}{+\text{-}(r)}$	$+\text{-}(r)\frac{+\text{-}(r)}{+\text{-}(r)}$	$\frac{Sb}{+\text{-}(r)}$	$+\text{-}(r)\frac{Cy\text{-}(r)}{+\text{-}(r)}$	$\frac{Sb}{+\text{-}(r)}$		$+\text{-}(r)\frac{+\text{-}(r)}{+\text{-}(r)}$	$\frac{+\text{-}(r)}{+\text{-}(r)}$
	Genotypes of the tested G_4 ♀♀ (paternal genomes)*								
Experiments	$\frac{+\text{-}(r)}{Cy\text{-}(r)}$ $+\text{-}(r)$	$\frac{+\text{-}(r)}{+\text{-}(r)}$	$\frac{+\text{-}(r)}{Sb}$	$\frac{+\text{-}(r)}{+\text{-}(r)}$	$\frac{+\text{-}(r)}{Cy\text{-}(r)}$ $+\text{-}(r)$	$\frac{+\text{-}(r)}{Sb}$	$\frac{+\text{-}(r)}{Cy\text{-}(r)}$ Sb	$\frac{+\text{-}(r)}{+\text{-}(r)}$	$\frac{+\text{-}(r)}{+\text{-}(r)}$
Ca	0/40	0/40	0/40	0/41	0/25	0/34	0/34	0/32	0/40
Cb	0/38	0/40	0/41	0/39	0/30	0/40	0/37	0/30	0/40
a	31/58	0/57	38/58	1/59	44/55	47/49	55/55	0/56	0/68
b	2/58	0/55	17/54	0/59	12/39	25/59	36/54	1/56	0/71

The numbers given are (number of sterile ♀♀)/(total number of tested G4 ♀♀).

* Since all the females come from *se* F5 mothers, their genotypes only differ by the genomes they have inherited from their fathers.

recombined chromosomes (two lower rows of histograms of Figure 5), 92 are sterile and 27 fertile in experiment (a) while 81 are sterile and 36 fertile in experiment (b). The frequencies of sterile females are not significantly different between the two experiments ($\chi^2 = 1.99$, d.f. = 1, $0.1<p<0,2$). On the contrary, among females carrying only the *w—(r)* and the *In(2L+2R)Cy—(r)* chromosomes (Figure 5, two upper rows), these frequencies are lower, since it was found 46 sterile and 51 fertile in experiment (a) and 7 sterile and 85 fertile in experiment (b). Moreover, the frequency obtained in the experiment (a) is significantly higher than that obtained in experiment (b) ($\chi^2 = 37$, d.f. = 1, $p<0,001$).

Therefore, the chromosomes *w—(r)* and *In(2L+2R)Cy—(r)*, from reactive origin may become inducer in spite of the use of crossing over inhibitors, and this genetic change occurs more frequently in *SF* than in *RSF* females. It is known that *In(1)*$sc^{s1L}sc^{s8R+S}$,$sc^{S1}sc^{s8}w^aB$ and *In(2L+2R)Cy* does not inhibit completely the crossing over throughout chromosomes *X* and *2* (LINDSLEY and GRELL 1967). Thus some *w—(r)* and *In(2L+2R)Cy—(r)* chromosomes might acquire exceptionally in the G_1 females some segments of the *In(1)* $sc^{s1L}sc^{s8R+S}$,$sc^{S1}sc^{s8}w^aB$*—(i)* and *+—(i)* chromosomes of the inducer stock and therefore become inducer by recombination. However, if such residual recombination can perhaps account for the 7 sterile G_3 females obtained in the experiment (b), it cannot account for the high number of sterile G_3 females obtained in the experiment (a). Moreover, residual recombination must be the same in *SF* and *RSF* G_1 females, since they have the same genotype, and this cannot explain the difference observed between the frequencies of sterile G_3 females in the experiments (a) and (b). It may be assumed that a change affecting the *w—(r)* and *In(2L+2R)Cy—(r)* chromosomes may occur, in *SF* females and perhaps also in *RSF* females, independently of the production of recombined gametes. This phenomenon is called chromosomal contamination. When recombination is not inhibited (because no chromosome *3* inversions are present), it seems to lead to an increase of the efficiency of this change and to a disappearance of any difference between *SF* and *RSF* females.

Evidence for the occurrence of a chromosomal contamination mechanism independent of the production of recombined gametes is brought forward quite clearly by the experiments of the second set. These experiments were designed to show whether contamination was limited to homologous chromosomes or could extend to nonhomologous. The *+—(i)/+—(i);In(2L+2R)Cy—(i)/Pm—(i);H—(i)/-Sb—(i)* inducer stock was used and it was shown that all the *Pm—(i)* and *Sb—(i)* chromosomes of this stock are inducer (i^+). It was also shown before that the chromosomes *4* of this stock are non-inducer. Four experiments were made on the same general scheme (Figure 6). They differ by the genotypes of the G_2 females which were used.

In the first experiment (a), G_2 females bearing only the *Pm—*(i^+) inducer chromosomes were chosen. In these females recombination is inhibited on the three major chromosomes. The chromosomes *In(1)*$sc^{s1L}sc^{s8R+S}$,$sc^{S1}sc^{s8}w^aB$*—(r)*, *In(2L+2R)Cy—(r)* and *In(3LR)DcxF—(r)* from reactive origin were recovered in the male offspring and assayed for their ability to carry *I* factor. For this purpose, the fertility was measured of about 40 to 50 females which had a re-

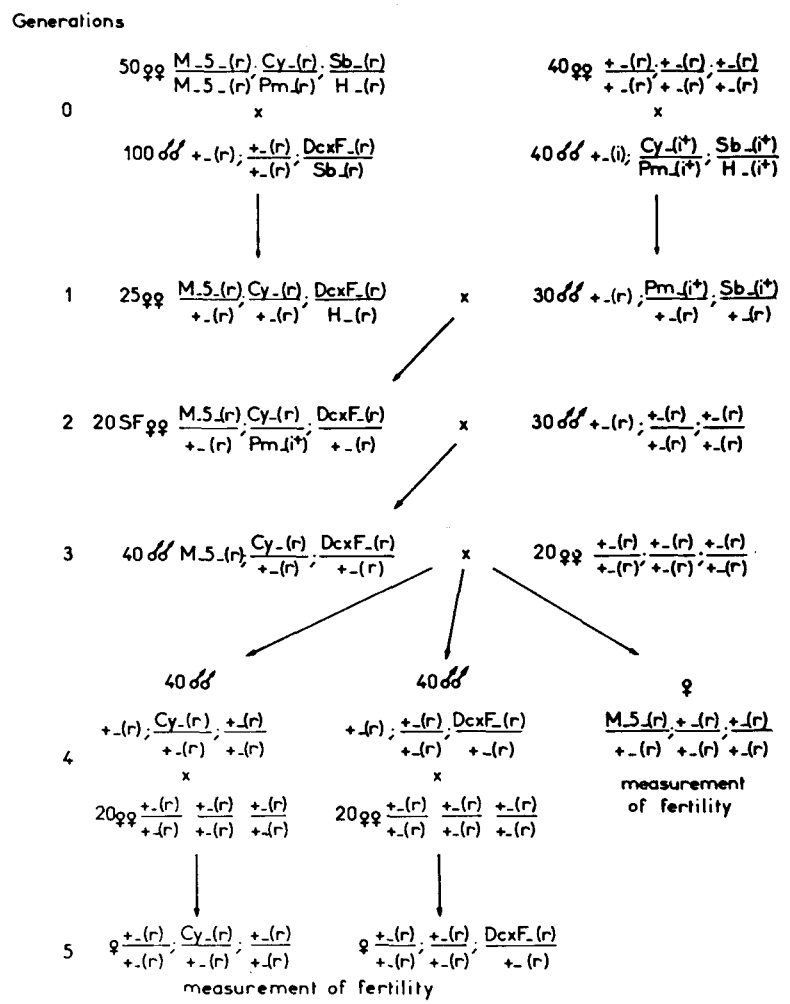

FIGURE 6.—Scheme of experiment (a)
M-5-(r) = *In(1) sc^{S1L}sc^{8R+S}, sc^{S1}sc^{8} w^{a} B-(r)*
Cy-(r) = *In(2L+2R)Cy-(r)*
DcxF-(r) = *In(3LR)DcxF-(r)*
The *+-(r)* chromosomes come either from the *+-(r)/+-(r); +-(r)/+-(r) In(3LR)-DcxF-(r)/Sb-(r)* reactive stock or from the *se* F_5 reactive stock.

active mother, and which had inherited from their father one of these three chromosomes. The finding of some sterile females would mean that the assayed chromosomes, or perhaps the chromosome *4*, carried by these females have been contaminated in the *SF* females.

In the second experiment (b), use was made in G_2 of *SF* females *+—(r)/-In(1)sc^{S1L}sc^{8R+S},sc^{S1}sc^{8}w^{a}B—(r);+—(r)/In(2L+2R)Cy—(r);Sb—(i^{+})/In-(3LR)DcxF—(r)*, which bear only the *Sb—(i^{+})* inducer chromosome. In the following generations, the same crosses and the same measurements were realized as in experiment (a).

In the third experiment (c), the G_2 *SF* females $+-(r)/In(1)sc^{S1L}sc^{s8R+S}$, $sc^{S1}sc^{s}$ w^{a} $B-(r)$; $Pm-(i^{+})/In(2L+2R)Cy-(r)$; $Sb-(i^{+})/In(3LR)DcxF-(r)$, bear both $Pm-(i^{+})$ and $Sb-(i^{+})$ inducer chromosomes.

The fourth experiment was designed to control the fertility of females bearing marked chromosomes, without involvement of *I* factor. The G_2 females $+-(r)/In(1)$ $sc^{S1L}sc^{s8R+S}$,$sc^{S1}sc^{s}w^{a}B-(r)$; $+-(r)/In(2L+2R)Cy-(r)$;$+-(r)/In(3LR)$ $DcxF-(r)$ do not bear any inducer chromosome.

Histograms of Figure 7 show the fertility of females bearing one of the three marked chromosomes $In(1)\ldots-(r)$, $In(2L+2R)Cy-(r)$ and $In(3LR)DcxF-(r)$, which have been present in the G_2 *SF* females with either one or two inducer chromosomes. They provide evidence for the following points.

1) Even when *SF* females were bearing only one inducer chromosome (the two upper rows (a) and (b) of the histograms), sterile females were found in the three genotypic classes. Therefore some chromosomes behave as inducer within

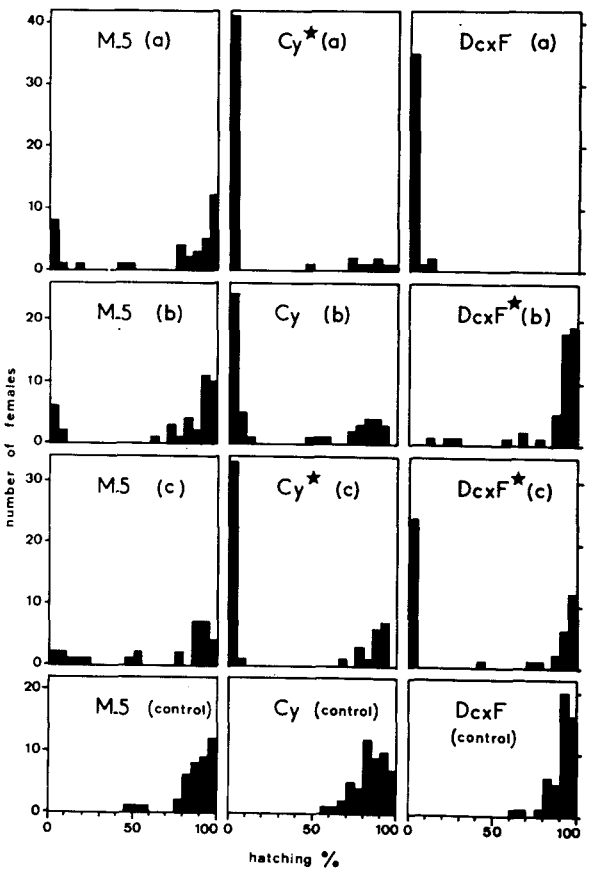

FIGURE 7.—Histograms show the fertility of the G_4 or G_5 females carrying one of the *M-5-(r)* = $In(1)$ $sc^{S1L}sc^{s8R+S}$, $sc^{S1}sc^{s}$ w^{a} *B-(r)*, $In(2L+2R)Cy-(r)$ or $In(3LR)DcxF-(r)$ chromosomes, obtained in each of the four experiments (a), (b), (c) and "control". The *Cy-(r)* and *DcxF-(r)* chromosomes are indicated by ★, when they have been homologous with the inducer chromosomes *Pm-(i)* or *Sb-(i)* in the G_2 *SF* females. (see text).

every one of the three classes. This result clearly indicates the occurrence of contamination between nonhomologous chromosomes.

2) Since the assayed chromosomes were not a sample of the chromosomes which were independently liable to become contaminated, but might include clones of various sizes, the available data do not allow a strict quantitative treatment. It seems, nevertheless, that among the chromosomes of reactive origin, those which were homologous of the inducer chromosomes were not more often contaminated than nonhomologues. Moreover, the presence of two inducer chromosomes in *SF* females does not seem to increase the number of sterile females (histograms (c) of Figure 7), and therefore the efficiency of contamination.

CONCLUSION AND DISCUSSION

The results presented in this paper provide evidence for a rather unusual genetic behavior of what has been called the *I* factor. As long as chromosomes of both origins are carried through successive generations of heterozygous males, *I* factor exhibits a strict Mendelian transmission, except that any of the three major chromosomes is a sufficient inducer. Indeed, the male gamete used to fertilize a female gamete from a reactive strain must include at least one chromosome from inducer origin to give rise to a sterile female.

When transmitted through successive generations of i^+/r heterozygous males, chromosomes from reactive origin never acquire the inducer character. A quite different genetic situation is met when chromosomes i^+ and r coexist in heterozygous *SF* females. Indeed, even when such females carry only a single i^+ chromosome, all the r chromosomes, even those which are nonhomologous with the i^+ chromosome, may become inducer. This phenomenon which therefore occurs when r chromosomes have no possibility of acquiring any segment from inducer origin by recombination has been denoted chromosomal contamination.

Up to the present time, it was indeed impossible to map *I* factor using heterozygous females bred from crosses between reactive and inducer strains, because in such females, contamination takes place. The finding of $2i^0$ chromosomes in the Luminy strain suggests an interesting possibility: if a polymorphism i^+/i^0 could be maintained in the Luminy strain, this indicates that the i^0 chromosomes cannot acquire *I* factor by contamination in females of this strain, and perhaps that contamination does not occur in the inducer strains. Using a chromosome $2i^+$ bearing some recessive markers, and the $2i^0$ Luminy chromosome it would be possible to map *I* factor by crosses involving only inducer strains. This possibility is presently under investigation.

The results reported in this paper do not allow a clear understanding of the identity of *I* factor. Chromosomal contamination bears at first sight some similarity with the phenomenon of paramutation at the *R* locus in maize (see review by BRINK 1973) and with the transpositions of controlling elements described in maize by several authors (see review by MCCLINTOCK 1965) but the available data are not sufficient to allow a detailed comparison with these phenomena. For instance chromosomal contamination may be viewed either as a translation of

genetic elements from one chromosome to another, or as some kind of derepression of genes carried by *r* chromosomes, which are inactive in reactive strains.

This work was supported by grants from the C.N.R.S.(R.C.P.149) and from the University of Clermont-Ferrand.

LITERATURE CITED

BRINK, R. A., 1973 Paramutation. An. Rev. of Genetics **7**: 129–152.

BUCHETON, A., 1973 Contribution a l'étude de la stérilité femelle non mendelienne chez *Drosophila melanogaster*. Transmission héréditaire des degrés d'efficacité du facteur *R*. C. R. Acad. Sc. Paris, D. **276**: 641–644.

BUCHETON, A. et G. PICARD, 1975 Mise en évidence d'une influence partiellement héritable de l'âge sur un phénomène de stérilité femelle à déterminisme non mendélien chez *Drosophila melanogaster*. C. R. Acad. Sc. Paris. D. **281**: 1035–1038.

DAVID, J., 1959 Etude quantitative du développement de la Drosophile élevée en milieu axénique. Bulletin de la Société Biologique de France et de Belgique **93**: No. 4, p. 472.

LINDSLEY, D. L. and E. H. GRELL, 1967 Genetic variations of *Drosophila melanogaster*. Carnegie Institution of Washington Publication No. 627.

MCCLINTOCK, B., 1965 The control of gene action in Maize. Brookhaven Symposia in Biology **18**: 162–184.

PICARD, G., 1971 Un cas de stérilité femelle chez *D. melanogaster*; lié à un agent transmis maternellement. C. R. Acad. Sc. Paris, D. **272**: 2482–2487.

PICARD, G., A. BUCHETON, J. M. LAVIGE et A. FLEURIET, 1972a Contribution à l'étude d'un phénomène de stérilité à déterminisme non mendelien chez *D. melanogaster*. C. R. Acad. Sc. Paris D. **275**: 933–936.

PICARD, G., A. BUCHETON, J. M. LAVIGE, A. FLEURIET, J. C. BREGLIAND and PH. L'HERITIER, 1972b Further data on nonmendelian female sterility in *D. melanogaster*. Dros Inf. Serv. **49**: 45.

PICARD, G. and PH. L'HERITIER, 1971 A maternally inherited factor inducing sterility in *D. melanogaster*. Dros. Inf. Serv. **46**: 54.

Corresponding editor: A. CHOVNICK