NON-MENDELIAN FEMALE STERILITY IN
DROSOPHILA MELANOGASTER: PRINCIPAL CHARACTERISTICS
OF CHROMOSOMES FROM INDUCER AND REACTIVE ORIGIN
AFTER CHROMOSOMAL CONTAMINATION

GEORGES PICARD

Laboratoire de Génétique, Université de Clermont-Ferrand II,
B.P. 45—63170 Aubière, France

Manuscript received April 3, 1978
Revised copy received October 6, 1978

ABSTRACT

Strains of Drosophila melanogaster can be divided into two main classes, inducer and reactive, in relation to non-Mendelian female sterility. The genetic element responsible for the inducer condition (I factor) is chromosomal and may be linked to any inducer-strain chromosome. Each chromosome carrying the I factor (i+ chromosome) can produce females showing more-or-less reduced fertility when it is introduced by paternal gametes into a reactive oocyte. As long as i+ chromosomes are transmitted through heterozygous males with reactive originating chromosomes (r chromosomes), I factor strictly follows Mendelian segregation. In contrast, in heterozygous i+/r females, a varying proportion of r chromosomes may acquire I factor independently of classical genetic recombination, by a process called chromosomal contamination. This paper reports investigation of the characteristics of the three kinds of chromosomes produced by females in which contamination occurs. It appears that the contaminated reactive chromosomes have irreversibly acquired I factor and behave like i+ chromosomes, while the i+ chromosomes used as contaminating elements and the reactive originating chromosomes that have not been contaminated have not undergone any change.

SYSTEMATIC crosses between strains of Drosophila melanogaster lead in some cases to poorly fertile F₁ females. On the basis of this phenomenon, strains can be divided into three classes: inducer, reactive and neutral (Picard et al. 1972). Crosses between strains within the same class and all crosses involving a neutral strain produce fertile F₁ daughters, while crosses between reactive females and inducer males lead to F₁ females, denoted SF females, which exhibit more-or-less reduced fertility. In contrast, reciprocal crosses between inducer females and reactive males produce normally fertile daughters, which have been denoted RSF females.

SF sterility results from the failure of some eggs to complete embryonic development; those hatching successfully give rise to flies that do not exhibit any conspicuous aberration. The hatching percentage does not depend on the mates of SF females and is therefore a purely maternal feature. A specific characteristic of this sterility is that hatchability rises regularly with the age of SF females.

Thus, it is always possible to recover progeny from SF females, even when they are entirely sterile at the beginning of their life (Picard et al. 1977). Even when measured on the first eggs laid by SF females, the hatching percentage is quite variable and may take any value from zero upwards, according to the choice of the reactive and, to a lesser degree, of the inducer parental strains (Bucheton et al. 1976).

The reactive strains may be arranged from strong to weak in relation to the reduction of fertility of F₁ females obtained from the cross of reactive females by males of a standard inducer strain. Females from strong reactive strains produce highly sterile SF females, while those from weak reactive strains give SF females showing only a slight reduction of fertility. A similar, although lesser, variability is found among inducer strains (Bucheton et al. 1976).

A survey of 171 strains has indicated that only the inducer condition is found in the wild, while strains belonging to the three classes are known in several European and American laboratories (Picard et al., 1976). Therefore, the inducer condition appears as the normal condition for Drosophila melanogaster, while the reactive and neutral conditions result from some as yet unexplained genetic change that has taken place in certain laboratory stocks. Neutral strains may be viewed as the extreme weak type of reactive strains, unable to give any noticeable SF sterility.

For the convenience of discussion, the genetic factors responsible for the reactive and inducer conditions have been denoted R and I, respectively. SF females result from the fertilization of an oocyte bearing a cytoplasm in a reactive state by a gamete carrying I factor. The available data on R factor clearly show that the reactive state tends to be maintained through maternal transmission, but is submitted, in the long run, to chromosomal control (Bucheton 1973; Bucheton and Picard 1975, 1978; Picard 1978a,b).

I is a chromosomal factor that may be linked to any of the three major chromosomes of inducer strains (Picard 1976) and even to the small fourth chromosome (Pellisson 1977). When heterozygous males bearing chromosomes from both inducer and reactive origins are mated with reactive females, they yield SF females only among the daughters that received at least one inducer originating chromosome carrying I factor. The chromosomes carrying I have been denoted i+ chromosomes (Picard 1976). However, all the chromosomes of inducer strains are not always i+. Some of them are unable to produce SF females when they are introduced by a paternal gamete into a reactive oocyte. They are, therefore, considered as not carrying I factor and are denoted i₀ (Picard 1976).

The Mendelian transmission of the I factor holds only when chromosomes from reactive origin (r) and i+ chromosomes are carried by males. Indeed, in SF and RSF females, r chromosomes may acquire I factor, often with a high frequency. This phenomenon, which occurs even for r chromosomes heterologous with the contaminating i+ chromosomes and therefore does not require classical genetic recombination, has been denoted “chromosomal contamination” (Picard 1976).
Several other cases of female sterility occurring in the F1 progeny of only one of the reciprocal crosses between various strains have recently been reported (Sved 1976; Kearsey et al. 1977; Kidwell, Kidwell and Sved 1977; Woodruff and Thompson 1977; Thompson and Woodruff 1978). Moreover, most of these authors observed that several aberrant traits including male sterility, recessive lethal mutation, male recombination, altered female recombination, segregation distortion, chromosomal aberration and nondisjunction are associated with female sterility. The name “hybrid dysgenesis” has been given to this group of related phenomena (Kidwell and Kidwell 1976; Sved 1976). The observations of these authors show many similarities with SF sterility, but also some clear differences. Studies at this time are being carried out with the collaboration of M. G. Kidwell in an attempt to clarify the relationships between hybrid dysgenesis and SF sterility.

Although the purpose of this paper is not to report these studies, the following observations can be made: There exists two kinds of female sterility. Indeed, the female sterility reported in Kidwell, Kidwell and Sved (1977) results mainly from a gonadal dysgenesis, and the classification established with this criterion is partly independent of the inducer-reactive classification established with the SF sterility criteria (Kidwell, in preparation).

It seems likely that male sterility is not associated with SF sterility. Indeed, even though this character has been systematically measured in only a few crosses, an abnormally high level of sterility among the male sibs of SF females has never been observed in our experiments. On the contrary, SF sterility is clearly associated with X-chromosome nondisjunction and high mutation rate, at least in females. These results will be published with a detailed discussion (Picard et al. 1979).

The available data do not permit one to decide whether the other dysgenic traits, especially male recombination (which has been widely investigated) (Voelker 1974; Waddle and Oster 1974; Slatko and Hiraizumi 1975; Kidwell and Kidwell 1976; Sochacka and Woodruff 1976; Woodruff and Thompson 1977), are or are not associated with SF sterility.

The purpose of this paper is to carry on the genetic analysis of SF sterility and of the phenomenon of chromosomal contamination that occurs in SF and RSF females. These females transmit to their offspring three kinds of chromosomes: the original \(i^+\) chromosomes, which have acted as contaminating elements in the females, and the \(r\) chromosomes, which may be either contaminated, if they have acquired \(I\), or not contaminated. These two kinds of \(r\) chromosomes are denoted \(rc^+\) and \(rc^0\), respectively. In this paper, I report experiments investigating the principal characteristics of these three kinds of chromosomes.

MATERIALS AND METHODS

Genetic symbols are those used by Lindsley and Grell (1968). Flies were grown on the axenic food described by David (1959) at 20°. Except when otherwise indicated, all crosses were made in mass.
Stocks of Drosophila melanogaster

**Reactive**

(1) seF$_s$ and seF$_g$ derive from the original se stock, homozygous for the mutation sepia. They were selected for a strong level of reactivity following the method described in Picard et al. (1972).

(2) LH$_{12}$ and LH$_{43}$ were selected in the same way from the original LH stock, the genotype of which is M-5/M-5; In(2L+2R)Cy, Cy/Pm; Sb/H. The X chromosome carries the inversion Muller-5 (= Basc, In(1) seB12seB+2S, seB12seB w$^B$ B). In(2L+2R)Cy bears the inversions In(2L)Cy and In(2R)Cy. These two chromosomes were used to suppress crossing over. (Pm = bw$^{v+}$.)

(3) DcxF/Sb-(R) comes from the Dept. of Genetics of Umeå University (Sweden). Its genotype is +/+; +/+; In(3LR)DcxF, D/Sb. The inversion In(3LR)DcxF was also used to suppress crossing over.

**Inducer:**

(1) Luminy is a wild-type strain coming from flies caught in 1969 in Southern France and maintained since then in our laboratory.

(2) Cy/Pm; H/Sb-(I) comes from the Genetics Laboratory of Lyon University (France). Its genotype is +/+; +/+; In(2L+2R)Cy, Cy/Pm; H/Sb. Only the chromosome 2 carrying In(2L+2R)Cy was used to suppress crossing over.

(3) DcxF/Sb-(I) comes from the Institute of Genetics of Stockholm University (Sweden). Its genotype is +/+; +/+; In(3LR)DcxF, ru h ca/In (3R)C Sb. Only In(3LR)DcxF was used to suppress crossing over.

All the stocks used in this work carry a wild-type fourth chromosome.

Symbols: Chromosomes originating from an inducer or a reactive stock are symbolized by the indices -(i) and -(r). The symbol -(rc) designates a -(T) chromosome which is (or which has been) carried by a female in which contamination takes place. It implies that the chromosome might have been contaminated with a certain probability and therefore it may be either rc$^+$ or rc$^-$.  

**Measurements of fertility**

**Individual females:** Two or three days after emergence, mated females were individually placed in culture vials containing food stained with carbon black. A sample of about 50 eggs was collected on this food over a period of about 48 hr. The eggs, which were easily seen on the black background, were recorded as hatched or not hatched 48 hr later. Females were mated with their brothers since it was shown that the hatching percentage of the eggs does not depend on the males used to fertilize SF females (Picard et al. 1977).

**Sets of females:** The measurements were made according to a similar method. The only differences were that about 20 females were allowed to deposit eggs collectively over a period of 24 hr and that each hatching percentage value was determined from a total of about 200 eggs.

**RESULTS**

**Characteristics of chromosomes from reactive origin that have been contaminated (rc$^+$)**

To allow a comparison between $i^+$ and rc$^+$ chromosomes, the two main characteristics of $i^+$ chromosomes (Picard 1976) have been studied for rc$^+$ chromosomes, i.e., stability of the linkage of I factor to rc$^+$ chromosomes in rc$^+/r$ heterozygous males and ability of rc$^+$ chromosomes to contaminate r chromosomes in rc$^+/r$ heterozygous females.

**Stability of the linkage of I factor to rc$^+$ chromosomes in successive generations of heterozygous rc$^+/r$ males:** The rc$^+$ or rc$^-$ character of the reactive originating chromosomes 2 and 3 from SF or RSF females cannot be tested without
FEMALE STERILITY IN DROSOPHILA

passing them through two successive generations of heterozygous males (Picard 1976); therefore, only those that have retained I factor can be detected as rc+. However, this observation does not demonstrate the stability of the linkage of I factor to rc+ chromosomes. Indeed, in the previous experiments (Picard 1976), the SF and RSF females produced both rc+ and rc− homologous chromosomes, and the hypothesis that the contaminated chromosomes progressively lose I factor in the generations following contamination cannot be ruled out. The stability of the linkage was checked by passing rc+ chromosomes through successive generations of rc+/r heterozygous males.

Two sets of experiments were performed with rc+ chromosomes contaminated in SF females, by either homologous or heterologous i+ chromosomes. In both sets, i+ chromosomes 2 of the Luminy inducer stock were used as contaminating elements. Since a polymorphism i+:io for chromosome 2 of the Luminy stock was previously suspected (Picard 1976), the following procedure was used to select i+ chromosomes 2.

Heterozygous males Basc-(r); Pm-(r)/+(i); H-(r)/+-(i+), from a mass cross between 20 LH12 reactive females and 25 Luminy males were individually mated with two Basc-(r)/+-(r); Cy-(r)/+-(r); Sb-(r)/se-(r) reactive females from a mass cross between the reactive stocks LH12 and seF5. In the progeny of each cross, the Basc-(r)/+-(r); Cy-(r)/+-(i); H-(r)/se-(r) daughters were recovered. Chromosomes 4 were not checked, but it was previously shown (Picard 1976) that the Luminy stock carry only io chromosomes 4. Therefore, these females, from a reactive mother were either SF females showing reduced fertility when they have received an i+ chromosome 2 from their father or were normally fertile when they have received an io chromosome 2.

The protocol of the first set of experiments is given in Figure 1. Twenty of these SF females, from a single male of generation one were mated with 20 seF5 reactive males. In the progeny, 25 males that had received from their SF mother the gamete +-(rc); Cy-(rc); H-(rc) were crossed with seF5 females. From generation four to generation nine, successive backcrosses were made between seF5

![Figure 1](image-url)
reactive females and males bearing the Cy-(rc) chromosome, which might be contaminated in SF females by the i+ homologous chromosome.

At generations five, eight and ten, the fertility of about 20 to 25 females carrying the Cy-(rc) chromosome, and the same number of females lacking it, was individually measured.

The same experiment was repeated starting from another male of generation one.

In both experiments, all the females of generations five, eight and ten that have inherited a Cy-(rc) chromosome from their father are entirely sterile, none of their eggs hatching. In contrast, females carrying only r chromosomes are normally fertile (hatching percentages higher than 70%), except for one that was entirely sterile (0%). This exceptional case has not been further investigated, and it is not known whether it bears any relation to SF sterility. These results indicate that in SF females of generation 2 contamination occurred with a high frequency, since in both experiments all the Cy-(rc) chromosomes tested at generation 5 are rc+. In addition, the linkage of I factor to these rc+ chromosomes is stable since it was maintained through seven successive generations of heterozygous rc+/r males.

The protocol of the first experiment of the second set (Figure 2) was similar to that of the first set and began also with a cross between 25 seF, males and 20 SF females from a single male of generation one. The chromosome tested in this case was the H-(rc) chromosome 3, which might be contaminated in SF females by heterologous i+ chromosome 2. Successive backcrosses to seF, females were made up to generation ten and at generations five, seven, nine and 11, the individual fertility of about 30 females carrying H-(rc) was measured.

This experiment was repeated twice, starting from two other males of generation one. However, in these two repeats, the Sb-(rc) chromosome was used instead of the H-(rc). This was achieved by using, at generation two, Sb-(rc)/se-(rc) SF females produced by a cross between individual Sb-(r)/+-se(r) males and two H-(r)/se-(r) reactive females.

**Figure 2.**—Mating scheme to test the stability of the inducer character of rc+ chromosomes through rc+/r heterozygous males. Case of rc+ chromosomes contaminated by heterologous i+ chromosomes. See legends in Figure 1. (M.5 in figure = Basc.)
The results are clear and corroborate entirely the conclusions of the preceding experiments. In the three experiments, whatever the generation of the test, all the females, without exception, laid eggs none of which hatched. Therefore, even when contaminated by \(i^+\) heterologous chromosomes, \(rc^+\) chromosomes keep their inducer character through at least eight successive generations of heterozygous \(rc^+ / r\) males.

**Contaminating activity of chromosomes** \(rc^+\) **in heterozygous** \(rc^+ / r\) **SF females:**

As \(i^+\) chromosomes, \(rc^+\) chromosomes induce sterility when they are brought by a paternal gamete into a reactive oocyte and keep this characteristic when they are transmitted by males. Two experiments were performed to determine whether \(rc^+\) chromosomes are also similar to \(i^+\) chromosomes in their ability to contaminate \(r\) chromosomes in \(SF\) females. They began respectively by the crosses of generation three of the two experiments of the first set previously described (see Figure 1). The experimental design is given in Figure 3.

At generation four, females that inherited from their father the chromosomes \(X+-(rc)\) and 2 \(Cy-(rc)\) were mated with \(seF_s\) males. These females coming from reactive mothers will be \(SF\) females if at least one of these two chromosomes is \(rc^+\). The purpose of this experiment is to determine whether contamination occurs in such \(SF\) females that do not carry any \(i^+\) chromosome. In the progeny of this cross, 15 males that did not bear the \(Cy-(rc)\) chromosome were recovered and mated with \(seF_s\) females. The fertility of about 25 daughters was individually measured. It must be noted that the protocol does not allow complete removal of the chromosomes that might have been contaminated in \(SF\) females of generation two. Indeed, the females of generation six may receive from their father the \(X+-(rc)\) chromosome, the \(X +-(r)\) or a product of their recombination.

As a control, at generation four, the individual fertility of about 35 females bearing the \(Cy-(rc)\) chromosome and of about 25 to 30 females that did not carry it was measured.

The results of the fertility measurements, which are given by the histograms of Figure 4, are very similar in both experiments. They indicate that all the females of generation four bearing the \(Cy-(rc)\) chromosome show a very reduced fertility. Therefore, it may be accepted that all the females of generation four bearing the \(Cy-(rc)\) chromosome are sterile.
Figure 4.—Contamination of r chromosomes by rc+ chromosomes in SF females. The two lines of histograms give respectively the results of the two experiments. Each square represents the fertility of a female. Histograms of the left and middle columns concern females of generation four carrying the chromosomes 2Cy-(rc) or +-(-r), respectively. Both classes of females bear a X +-(rc) chromosome. Histograms of the right column concern females of generation six used in the matings carry at least one rc+ chromosome and are SF females. In contrast, among females of generation four that bear a 2 +-(r) chromosome, only a few show low fertility. This means that only a few of the X +-(rc) chromosomes have been contaminated in SF females of generation two. Therefore, the presence of X +-(rc) chromosomes in statistically half of the females of generation six cannot account for the large number of sterile females observed (histograms of the right column). Most of the females of generation six exhibit very low fertility and therefore have received at least one rc+ chromosome from their father. It may be concluded that in SF females of generation four, a certain proportion of +-(r) or se-(r) chromosomes have been contaminated by rc+ chromosomes.

Characteristics of chromosomes from reactive origin that have escaped contamination (rco)

Except in some cases in which contamination seems to occur with a frequency of 100%, rc o chromosomes may be recovered in the progeny of SF or RSF females. These chromosomes are unable to produce SF females when introduced by a paternal gamete into a reactive oocyte and are therefore considered as not having acquired I factor. An experiment was carried out to show whether rc o chromosomes may be contaminated when they are reintroduced into a female in which contamination occurs.

rc o chromosomes were sought in the progeny of RSF females since it is known that the efficiency of contamination is lower in these females than in SF females (Picard 1976). RSF females Basc-(rc)/+-(i+); Cy-(rc)/+-(-i); DcxF-(rc)/
+-(i^+) were produced by a cross between females of the inducer stock Luminy and reactive males Basc-(r); Cy-(r)/+-(r); DcxF-(r)/H-(r) from a cross between the reactive stocks LH_{12} and DcxF/Sb-(R). These RSF females carry on each of the three major chromosomes several inversions that strongly inhibit the production of recombined gametes. Several matings were made to test the rc^+ or rc^o character of Cy-(rc) chromosomes.

For this purpose, RSF females were individually mated with seF_s reactive males. In the progeny of each cross, +-(i^+); Cy-(rc)/+-(r); DcxF-(rc)/se-(r) males were mated with seF_s females and gave rise to +-(r); Cy-(rc)/+-(r); se(r)/se-(r) males, which were mated with seF_s females. The individual fertility of daughters that inherited the Cy-(rc) chromosome from their father was then measured. According to the rc^+ or rc^o character of their Cy-(rc) chromosome, these females were either sterile or fertile. Five fertile females giving hatching percentages higher than 80% were recovered in the progenies of three RSF females (number 2, 5 and 9), which produced a high proportion of sterile females.

Figure 5 gives the successive crosses to introduce the Cy-(rc^o) chromosome of each of the five fertile females into SF females and then to test its inducer or noninducer character. The five females of generation zero carried only chromosomes of reactive origin and were directly inherited from a reactive mother. They behaved as reactive females, since when crossed with Luminy inducer males they gave rise to SF females showing very reduced fertility. In the progeny of SF females, the Cy-(rc) chromosomes were recovered in males. Two successive backcrosses to seF_s reactive females were necessary to remove all other chromosomes that might have been contaminated in SF females. At generation four, the fertility of about 20 females bearing Cy-(rc) and of 20 females that do not bear it was measured individually. The first measurement indicates the inducer or noninducer character of the Cy-(rc) chromosomes and the second is a control.

---

**Figure 5.**—Mating scheme for contaminating the Cy-(rc^o) chromosomes. The inducer males of the original cross come from the Luminy stock. The reactive females used at generations two and three come from the seF_s stock. At generation 1, males originated from the LH_{23} stock. (M.5 in figure = Basc.)
Results are given in Figure 6. They show that almost all the females that do not carry \( C_y-(rc) \) have a normal fertility. Nevertheless, few of them exhibit a very reduced fertility in spite of the fact that they bear three major chromosomes of reactive origin that cannot be contaminated. It may be supposed that these sterile females have inherited from their father a chromosome 4 that was contaminated in SF females. Whatever the case, among females bearing \( C_y-(rc) \), a far larger proportion show very reduced fertility. This means that these females carry a \( C_y-(rc^+) \) chromosome and therefore that in SF females, \( C_y-(rc^o) \) chromosomes have been contaminated.

*Characteristics of \( i^+ \) chromosomes that were the contaminating elements in SF females*

From the experimental evidence reported above, it appears that chromosomal contamination involves an irreversible change in \( r \) chromosomes, which may occur in SF or RSF females with variable frequencies. It may be asked whether this phenomenon also causes some change in the \( i^+ \) chromosomes that have acted as contaminating elements. An experiment was performed in an attempt to provide answers to the following questions: Do the \( i^+ \) chromosomes retain their inducer character? and, do the \( i^+ \) chromosomes retain their ability to contaminate \( r \) chromosomes when they are reintroduced into SF females?

This experiment consists of transmitting \( i^+ \) chromosomes through successive generations of \( i^+/r \) heterozygous females in which contamination occurs and

![Figure 6](#)

*Figure 6.*—Contamination of \( C_y-(rc^o) \) chromosomes. Each square gives the fertility of a female taken from the progeny of each of the five fertile females of generation 0, 2a, 2b, 5a, 5b and 9a. Histograms of the left and right columns concern females of generation four without and with \( C_y-(rc) \), respectively.
measuring at various generations the inducer character of the $i^+$ chromosomes. After passing through seven successive heterozygous females, the $i^+$ chromosomes were first recovered in males bearing only noncontaminated reactive originating chromosomes, and then were introduced into SF females in which their ability to act again as contaminating elements was checked.

For the sake of clarity, the experiment was divided into three parts, which will be described successively.

**Transmission of Cy-$(i^+)$ chromosomes through successive $i^+/r$ females:** The mating scheme is given in Figure 7. Two crosses were made to produce SF females carrying a single $i^+$ chromosome, the Cy-$(i^+)$ chromosome of the Cy/Pm; H/Sb-(I) inducer stock. In the following generations, this chromosome was transmitted from mother to daughter up to generation nine, in the presence of chromosomes from reactive origin. At generations two, four, six and eight, males originated from the DcxF/Sb-(R) stock, while at generations three, five, seven and nine they originated from the seF$_s$ reactive stock. Thus, DcxF-$(r)$ chromosomes can be contaminated only at a single generation. They were used to measure the frequency of contamination.

Since contamination is known to occur in SF females and also in their female progeny (Picard 1978a,b), this protocol gives to Cy-$(i^+)$ chromosomes the highest possible probability to be used as contaminating elements. However, it must be noticed that a decrease in frequencies of contamination has to be expected from generation two to generation nine. Indeed, it is known (Picard 1978c) that the frequency of contamination is correlated with the level of fertility of the females in which it occurs; the lower the fertility, the higher the frequency of contamination. Now, it was shown (Picard 1978a,b) that in the female progeny

\[\text{Figure 7.} - \text{Transmission of a Cy-$(i^+)$ chromosome through successive generations of heterozygous $i^+/r$ females. The inducer males of the original cross are from the Cy/Pm; H/Sb-(I) stock. It was shown (Picard 1976) that chromosomes 2 and 3 are $i^+$. No data are available about the X chromosome. Females of the original cross are from the seF$_s$ stock, as are males used at the generations one, three, five, seven and nine. Males of generations two, four, six and eight are from the DcxF/Sb-(R) stock. Only chromosomes Cy-$(i^+)$ and DcxF-$(r)$ carry inversions that strongly inhibit recombination.}\]
of SF females, the cytoplasm cannot be maintained in a reactive state when $i^+$ chromosomes are present and therefore that the fertility is re-established after some generations. Although female fertility was not systematically measured at each generation, an increase of fertility was observed from generation two to generation nine. The SF females of generation two were highly sterile, the hatching percentage of the eggs being 0% in the first layings. Females of generations three to eight were more fertile since an increasing proportion of larvae was observed in the first layings. Lastly, at generation nine, the individual fertility of about 40 females was measured; all the hatching percentages were found to be higher than 60%, the majority of them exceeding 80%.

Test of the inducer character of Cy-$(i)^+$ and of the frequency of contamination: The inducer character of Cy-$(i^+)$ was tested from chromosomes recovered from females of generations two, three, five, seven and nine. As a control, frequencies of contamination were measured by testing the inducer character of $X+(rc)$ chromosomes issued from SF females of generation two and of $DcxF-(rc)$ chromosomes issued from females of generation three, five, seven and nine.

The matings schemes are presented in Figure 8. They are slightly different according to the chromosomes studied. However, the principle of the tests is the same. It consists in measuring the fertility of daughters of reactive mothers.

![Mating scheme for the test of the inducer character of Cy-$(i^+)$ and for the measurement of the frequency of contamination.](image)

**Figure 8.**—Mating scheme for the test of the inducer character of Cy-$(i^+)$ and for the measurement of the frequency of contamination. The original crosses are those represented in Figure 7, at the corresponding generations. The reactive females used for the test come from the se$F_8$ stock. The numbers of males $(n)$ recovered in the progeny of females of generations three, five, seven and nine are 39, 39, 36 and 38, respectively.
which have inherited from their father a gamete bringing the chromosome studied and chromosomes from reactive origin which cannot be contaminated.

The first set of crosses was made to test the inducer character of $38\, Cy-(i^+)$ and $38\, X\, +-(rc)$ chromosomes coming from SF females of generation two (upper part of Figure 8). In the progeny of these females, 38 males that have received a $Cy-(i^+)$ chromosome from their mother were individually mated with two $seF_s$ females. The fertility of a set of about 20 daughters carrying a paternal gamete $+-(rc); +-(r); DcxF-(r)$ was measured for each cross. In addition, in each case four males carrying $Cy-(i^+)$ and $DcxF-(r)$ were recovered and mated with five $seF_s$ females, and the fertility of a set of about 20 daughters bearing the paternal gamete $+-(r); Cy-(i^+); +-(r)$ was measured.

The second set of crosses was made to test the inducer character of $39\, Cy-(i^+)$ and $39\, DcxF-(rc)$ chromosomes coming from females of generation three (lower part of Figure 8). 39 males bearing both of these chromosomes were individually mated with two $seF_s$ females. As previously, in the progeny of each cross, four males carrying both $Cy-(i^+)$ and $DcxF-(rc)$ were recovered and mated with $seF_s$ females. In each case, the fertility of a set of about 20 females bearing $Cy-(i^+)$ and of about 20 females bearing $DcxF-(rc)$ was measured.

Three other sets of crosses were made following the same protocol to test the inducer character of chromosomes $Cy-(i^+)$ and $DcxF-(rc)$ originating respectively from females of generations five, seven and nine.

As expected, the results of the fertility measurements of females carrying $X\, +-(rc)$ or $DcxF-(rc)$ chromosomes indicate a decrease of the frequency of contamination from generation two to generation three.

All of the $38\, X\, +-(rc)$ chromosomes coming from SF females of generation two were found to be $rc^+$. Since it is known (Picard 1978c) that $X$ chromosomes show a lower ability to be contaminated than chromosome 2 and probably than chromosome 3, it can be assumed that most, if not all, of the reactive originating chromosomes have been contaminated. Therefore, in females of generation two, in which the $Cy-(i^+)$ chromosomes are the only possible contaminating elements, contamination occurred with a maximum efficiency. In females of following generations, contamination occurred with a lower efficiency since the proportions of $rc^+$ chromosomes among the $DcxF-(rc)$ chromosomes coming from females of generations three, five, seven and nine are $12/39, 1/39, 4/36$ and $4/38$, respectively. In these females, both the $Cy-(i^+)$ chromosomes and the $rc^+$ chromosomes contaminated in preceding generations might, a priori, be used as contaminating elements.

The results of the fertility measurements of females carrying $Cy-(i^+)$ chromosomes are clear. In the whole experiment, all the sets of 20 females are entirely sterile, except four that give hatching percentages lower than 4%. Therefore, it can be concluded that the $Cy-(i^+)$ chromosomes recovered from the females of various generations have kept their inducer character.

Test of the ability of $Cy-(i^+)$ chromosomes produced by the $i^+/r$ females of generation nine to contaminate $r$ chromosomes: In females, from generation three to nine, the contaminating elements may, a priori, be the $Cy-(i^+)$ chromo-
somes, but also any other reactive originating chromosome contaminated in females of preceding generations. Therefore, the measurements of the frequency of contamination in these females do not provide information as to whether or not the Cy-(i+) chromosomes have kept their ability to contaminate r chromosomes. For this purpose, Cy-(i+) chromosomes transmitted by females of generation nine were recovered and introduced into SF females in which they were the only possible contaminating elements.

It was indicated above that the inducer character of 38 of these chromosomes was tested by measuring the fertility of 38 sets of about 20 ++(r); Cy-(i+)/+(r); se-(r)/se-(r) females. It was also noted that these females were sterile at the beginning of their life. Since they come from a reactive mother, they were SF females in which contamination must occur if the Cy-(r) chromosomes have retained their ability to contaminate r chromosomes.

Fifteen of these females were taken at random in the 38 sets and mated with seF, males. In the progeny, 20 males ++(ir);++(ir); se-(rc)/se-(r) were crossed with 50 seF, females and the fertility of 75 daughters was individually measured.

The results indicate that of 75 females, only seven showed normal fertility (hatching percentages higher than 80%). The 68 other females were sterile, the hatching percentages of their eggs being 0%, except in one case in which it reached 3%. Therefore, 68 females inherited from their father at least one r+ chromosome. This signifies that contamination had occurred in the 15 females carrying Cy-(i+) and consequently that even after being transmitted through seven successive generations of females in which contamination occurred with variable efficiencies, these chromosomes have kept the ability to contaminate.

CONCLUSION AND DISCUSSION

The experiments reported in this paper have permitted determination of the main characteristics of the three kinds of chromosomes transmitted by females in which chromosomal contamination occurs:

Chromosomes from reactive origin that have not been contaminated (rco) do not exhibit any genetic resistance to contamination, since they can be contaminated when reintroduced into females in which contamination occurs. This observation provides direct evidence that the segregation of r+ from rco observed in the progeny of i+/r heterozygous females does not reflect some kind of polymorphism of r chromosomes for their ability to be contaminated, but results from a chance event. Moreover, it indicates that rco chromosomes have not undergone any detectable change, since they have kept the two main characteristics of reactive originating chromosomes with respect to I factor: noninductibility and ability to be contaminated.

When they are contaminated, chromosomes from reactive origin (r+) behave as inducer chromosomes: (1) They give rise to sterile females when they are brought by a paternal gamete into a reactive oocyte. (2) They retain this char-
acteristic even when they are transmitted through several successive generations of heterozygous \( rc^+ / r \) males. The same stability occurs when \( rc^+ \) chromosomes have been contaminated by a heterologous or by a homologous \( i^+ \) chromosome. (3) They are able to contaminate \( r \) chromosomes in heterozygous \( rc^+ / r \) females. Therefore, chromosomal contamination appears as a chance event that may befall any chromosome from reactive origin when it coexists in a female with chromosomes carrying the \( I \) factor. It involves an irreversible genetic change of the chromosome, which acquires the main characteristics of an \( i^+ \) chromosome.

Nevertheless, it cannot be asserted from the available data that \( rc^+ \) chromosomes are in all points identical to \( i^+ \) chromosomes. Indeed, it was shown (Bucheton and Picard 1978) that \( r \) chromosomes are necessary for the maintenance of a cytoplasm in a reactive state. In contrast, \( i^+ \) chromosomes bring about the loss of reactive character of the cytoplasm (Picard 1978a,b). This was illustrated in the last experiment described in this paper (Figure 7) in which it appears that a single \( i^+ \) chromosome transmitted through successive generations of females is sufficient to cause this effect. Indeed, \( SF \) females of generation two that come from reactive mothers are sterile, while seven generations later, females of the same genotype show almost normal fertility. There is at present no data concerning the effect of \( rc^+ \) chromosomes on the maintenance of the reactive state. Some observations (Picard 1978b) seem to indicate that they behave rather as \( i^+ \) than as \( r \) chromosomes, but it cannot be asserted that they are, on this point, similar to \( i^+ \) chromosomes.

The inducer chromosomes (\( i^+ \)) recovered from \( i^+ / r \) females in which contamination occurs do not exhibit any conspicuous change: they keep their inducer character and their ability to contaminate \( r \) chromosomes. Therefore, in \( i^+ / r \) heterozygous females, as well as in males, \( i^+ \) chromosomes remain permanently associated with \( I \) factor.

However, it cannot be strictly asserted that the \( i^+ \) chromosomes recovered in this experiment derive from those that have effectively acted as contaminating elements, since the possibility remains that the act of contamination leads to dominant lethality. This hypothesis, however, is not very likely even if at this time it cannot be ruled out. Indeed, it requires that contamination can occur between different germ cells since all the gametes recovered from \( SF \) females of generation two carry both an \( X^+ - (rc^+) \) and a \( Cy^+ - (i^+) \) chromosome. Moreover, it would probably lead to a segregation distortion in the progeny of \( i^+ / r \) females, and it is known (Picard et al. 1978) that this is not the case, even for \( SF \) females showing a high reduction of fertility in which contamination is expected to occur with a high efficiency. Therefore, it may be concluded, at least as a first approximation, that most of the \( Cy^+ - (i^+) \) chromosomes recovered in the progeny of females of various generations derive from chromosomes that have acted at least one time as contaminating elements. This means that the mechanism(s) of contamination do(es) not involve the loss of \( I \) factor by the \( i^+ \) chromosomes.

The results reported in this paper do not permit a clear understanding of chromosomal contamination. This phenomenon seems to be unique, although
it shows some similarities with paramutation at the \( R \) locus in maize (see review by Brink 1973). With the present evidence, two main hypotheses may be suggested. First, contamination might be only a derepression of genes carried by every chromosome of all classes of strains, but inactive in reactive strains. Second, it might be the result of the insertion in \( r \) chromosomes of transposable genetic element(s) carried by \( i^+ \) chromosomes. In this hypothesis, the invasion of \( r \) chromosomes must occur without loss of the genetic element(s) by the \( i^+ \) chromosomes, since after contamination they keep all their characteristics. Finally, it may be noted that these hypotheses are not mutually exclusive since it was shown in maize that the expression of several genes is under the control of genetic elements that can transpose from one site to one another (see reviews by Fincham and Sastry 1974 and by Nevers and Saedler 1977).

I am grateful to J. C. Brecciano and Ph. L’Heritier for advice throughout this work and for helpful comments on the manuscript. This work was supported by grants from the C.N.R.S. (ERA 692: Phénomènes d’hérédité non-mendelienn chez la Drosophile) and from the University of Clermont-Ferrand II.

**LITERATURE CITED**

Brink, R. A., 1973  

Bucheton, A., 1973  


Bucheton, A. and G. Picard, 1975  

David, J., 1959  


Kidwell, M. G. and J. F. Kidwell, 1976  

Hybrid dysgenesis in *Drosophila melanogaster*: a syndrome of aberrant traits including mutation, sterility and male recombination. Genetics 86: 813–833.

Lindsley, D. L. and E. H. Grell, 1978  

Nevers, P. and H. Saedler, 1977  
FEMALE STERILITY IN DROSOPHILA


