NON-MENDELIAN FEMALE STERILITY IN *DROSOPHILA MELANOGASTER*: CHARACTERIZATION OF THE NONINDUCER CHROMOSOMES OF INDUCER STRAINS

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

ABSTRACT

In relation to non-Mendelian female sterility, *Drosophila melanogaster* strains can be divided into two main classes, inducer and reactive. 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, when introduced by the paternal gamete into a reactive oocyte, give rise to females (denoted *SF*) showing more-or-less reduced fertility. As long as $i+$ chromosomes are transmitted through heterozygous males with reactive originating chromosomes (*r* chromosomes), *I* factor follows Mendelian segregation patterns. In contrast, in heterozygous $i+/r$ females, a varying proportion of *r* chromosomes may irreversibly acquire *I* factor, independently of classical genetic recombination, by a process called chromosomal contamination. The contaminated reactive chromosomes behave as $i+$ chromosomes.——In the present paper, evidence is given that the Luminy inducer strain displays a polymorphism for two kinds of second chromosomes. Some of them are $i+$, while others, denoted i^o, are unable to induce any *SF* sterility when introduced by paternal gametes into reactive oocytes. They are also unable to induce contamination of *r* chromosomes, but, like *r* chromosomes, they may be contaminated by $i+$ chromosomes in *SF* or *RSF* females. The study of the segregation of $i+$ and i^o second chromosomes in the progeny of heterozygous Luminy males and females leads to the conclusion that on chromosome *2* of the Luminy stock the *I* factor is at a single locus. ——*X*, second and third i^o chromosomes have been found in several inducer strains. Since these chromosomes can be maintained with $i+$ chromosomes in inducer strains in spite of their ability to be contaminated in *RSF* females, it can be concluded that chromosomal contamination does not take place in females of inducer strains. This implies that contamination occurs only in cells having cytoplasm in a reactive state.

SYSTEMATIC crosses between strains of *Drosophila melanogaster* lead in some cases to poorly fertile F_1 females. On the basis of the fertility of F_1 progeny, three classes of strains, inducer, reactive and neutral, may be recognized. Crosses between reactive females and inducer males produce F_1 females (symbolized *SF*) that may show a more-or-less reduced fertility, while reciprocal crosses between inducer females and reactive males give only fertile females (denoted *RSF*). Crosses between strains within the same class or crosses involving

a neutral strain give fertile F_1 females (PICARD *et al.* 1972; PICARD *et al.* 1976). For the convenience of experimental analysis, the genetic factors responsible for the reactive and inducer condition have been denoted *R* and *I*, respectively. Both of them exhibit non-Mendelian behavior. The principal characteristics of *SF* sterility and the available data relevant to hereditary transmission of *R* and *I* are mentioned in the introduction of the preceding paper.

Several authors have recently reported cases of female sterility that occur nonreciprocally in the F_1 progeny of crosses between certain strains and which are generally associated with one or several aberrant traits including male sterility, male recombination, alteration of female recombination, high mutation rate, chromosome aberration, nondisjunction and segregation distortion (SVED 1976; KEARSEY *et al.* 1977; KIDWELL, KIDWELL and SVED 1977; WOODRUFF and THOMPSON 1977; THOMPSON and WOODRUFF 1978). The name "hybrid dysgenesis" has been given to this related group of phenomena (KIDWELL and KIDWELL 1976; SVED 1976). *SF* sterility shows many similarities to the observations reported by these authors, but also some clear differences. It was recently demonstrated (KIDWELL, in preparation) that two partly independent systems are involved. Some recent observations concerning this point have been mentioned in the introduction of the preceding paper.

The purpose of the present paper is to carry on the genetic analysis of *I* factor. It was shown previously (PICARD 1976) that *I* is a chromosomal factor that may be linked to any of the chromosomes of inducer strains. Each chromosome bearing the *I* factor can give rise to *SF* females when introduced by a paternal gamete into reactive oocytes. These inducer-strain chromosomes carrying *I* have been called inducer chromosomes (i^+). However, all the chromosomes of inducer strains are not always i^+; some of them are unable to produce *SF* females when introduced by a paternal gamete into a reactive oocyte. Such noninducer chromosomes have been considered as not carrying *I* factor and are denoted i^0 (PICARD 1976). In this earlier paper, it was shown that chromosome *4* of several inducer stocks is i^0, and it was suspected that there is a polymorphism for i^+ and i^0 for chromosome *2* in the Luminy inducer stock. It should be noted that in their studies on a case of female sterility, which is probably the same, KEARSEY *et al.* (1977) demonstrated the existence of such a polymorphism for both chromosomes *2* and *3* of two laboratory populations.

In this paper, the existence of a polymorphism for chromosome *2* of the Luminy stock is demonstrated, and the characteristics of i^0 chromosomes are investigated especially regarding chromosomal contamination. The principal conclusions that may be drawn from the data are, first, that contamination does not occur in inducer females and, second, that on i^+ Luminy chromosome *2*, *I* factor is located at only one locus. Furthermore, evidence is brought forward for the existence of *X*, *2* and *3* i^0 chromosomes in stocks coming from flies caught recently in the wild.

MATERIALS AND METHODS

See the preceding paper (PICARD 1979).

RESULTS

Evidence for the existence of two genetic types of Luminy chromosome 2

In a previous paper (PICARD 1976; Figure 1), it was shown that females that come from strong reactive mothers and have received a paternal gamete bearing chromosomes *X* and *3* from reactive origin and a chromosome *2* originating from the Luminy inducer stock belong to two well defined classes: some of them show a strong reduction of fertility, while the others are normally fertile. This heterogeneity may *a priori* mean either that each Luminy chromosome *2* induces sterility, but only with some frequency, or that two genetic types of chromosome *2*, inducer (i^+) and noninducer (i^o), coexist in the Luminy stock. To make a choice between these two hypotheses, the following experiment was carried out.

An initial cross between 40 females *Basc-(r)/Basc-(r)*; *Cy-(r)/Pm-(r)*; *H-(r)/Sb-(r)* of the reactive stock LH_{12} and 60 males *+-(i+)*; *+-(i)/+-(i)*; *+-(i+)/+-(i+)** of the inducer stock Luminy bred heterozygous males *Basc-(r)*; *Pm-(r)/+-(i)*; *H-(r)/+-(i+)*. Twenty-eight of these males were individually mated with two females *Basc-(r)/+-(r)*; *Cy-(r)/+-(r)*; *Sb-(r)/se-(r)* produced from a cross between the reactive stocks, LH_{12} and seF_5. In the progeny of each cross, six males *+-(r)*; *Cy-(r)/+-(i)*; *H-(r)/se-(r)* of generation two were recovered and mated with ten seF_5 reactive females. In each case, the individual fertility of 31 daughters *+-(r)/+-(r)*; *+-(r)/+-(i)*; *se-(r)/se-(r)* was measured. Thus, all the females of generations three (G_3) that came from a same cross have inherited from their father *+-(i)* chromosomes *2* that constitute a single clone derived from a unique *+-(i)* chromosome *2* carried by the original male of generation one (G_1). Therefore, within the progeny of a unique G_1 male, the first hypothesis predicts that both classes of G_3 females (with normal or reduced fertility) will be observed, while the second hypothesis predicts that all the G_3 females will belong to the same class.

The results given in Table 1 are in agreement with the second hypothesis. In

TABLE 1

Fertility of the G_3 females coming from each of the 28 G_1 males

Number of G_1 males		16	3	9								
Hatching percent of the eggs laid by G_3 females	0–5%	499	0	0	0	0	0	0	0	0	0	1
	5–20%	0	0	0	0	0	0	0	0	0	0	0
	20–40%	0	0	0	0	0	0	0	0	1	1	0
	40–60%	0	0	0	0	0	0	1	1	0	1	0
	60–80%	0	0	1	1	2	3	1	1	2	6	4
	80–100%	0	96	31	31	29	28	29	29	28	23	26

For each sample of about 31 G_3 females derived from each of the 28 G_1 males, the table gives the distribution of the females according to their fertility. When several G_1 males give G_3 females all of which belong to the same class of fertility, the data are pooled and the number of G_1 males is indicated in the upper line.

* It was shown (PICARD 1976) that in the Luminy stock, chromosomes *X* and *3* are i^+ and chromosome *4* is i^o.

16 samples of about 31 G_3 females, all the females, without exception, show highly reduced fertility, since the hatching percentages do not reach more than 5%. Conversely, in three samples, all the females give hatching percentages higher than 80% and may be considered as normally fertile. In the nine other samples, a majority of G_3 females give hatching percentages higher than 80% but a few of them show a more-or-less reduced fertility. These females have not been studied further; therefore, we do not know whether their reduction of fertility bears any relation to the sterility phenomenon investigated in this paper. In spite of the existence of such G_3 females, it may be admitted that two genetic types of chromosome *2*, *+-(i+)* and *+-(i°)*, coexist in the Luminy inducer stock. However, the hypothesis that *+-(i°)* chromosomes may in some exceptional cases induce some reduction of fertility cannot be ruled out.

Ability of +-(i°) *Luminy chromosomes* 2 *to act as contaminating elements*

An experiment was carried out to determine whether *i°* chromosomes can induce contamination of *r* chromosomes in females from reactive origin. This experiment started from the progenies of 16 of the 28 G_1 males *Basc-(r)*; *Pm-(r)/+-(i)*; *H-(r)/+-(i+)* of the previous experiment. These males came from a cross between LH_{12} reactive females and Luminy inducer males and were individually mated with two *Basc-(r)/+-(r)*; *Cy-(r)/+-(r)*; *Sb-(r)/se-(r)* reactive females. Among the 16 G_1 males used in this experiment, 12 were those previously detected as carrying a *+-(i°)* chromosome *2* and four were taken at random among those that had been found to bear a *+-(i+)* chromosome *2*.

The matings made from each of these 16 progenies are given in Figure 1. The females of generation two (G_2) recovered in each progeny come from reactive mothers and carry major chromosomes from reactive origin, except for their

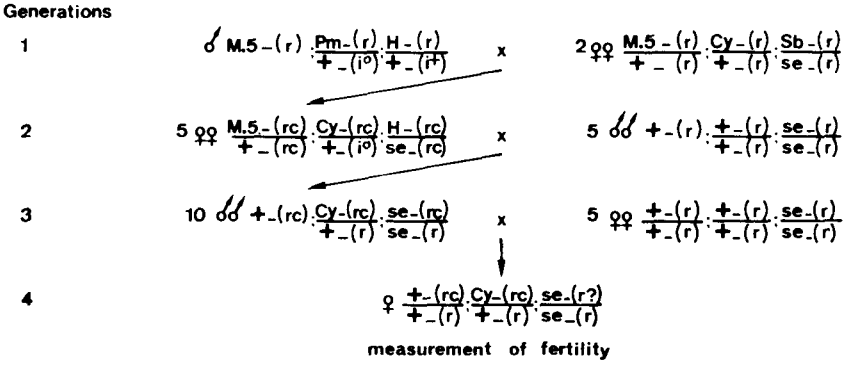

FIGURE 1.—Mating scheme to test the ability of *+-(i°)* chromosomes *2* to induce contamination. The same matings were made starting from 16 of the 28 males of generation 1 (see text). These males come from a mass cross between 40 females *Basc-(r)/Basc-(r)*; *Cy-(r)/Pm-(r)*; *H-(r)/Sb-(r)* of the reactive stock LH_{12} and 60 males *+-(i+)*; *+-(i)/+-(i)*; *+-(i+)/+-(i+)* of the Luminy inducer stock. Females of generation one come from a cross between the LH_{12} and *se*F_5 reactive stocks. Males of generation two and females of generation three are from the *se*F_5 strong reactive stock. The chromosome bearing *Cy* = *In(2L+2R)Cy* is used to suppress crossing over. (M.5 in the figure = *Basc*, *i.e.*, Muller-5).

paternally inherited +-*(i)* chromosome *2* which, according to the G_1 male, may be either i^+ or i^0. In four progenies, the females were *SF* females showing reduced fertility, while in the 12 other progenies, they were fertile females. In order to determine whether contamination takes place in G_2 females, some of their reactive originating chromosomes (*rc*) were tested for the inducer character. For this purpose, the individual fertility of G_4 females that came from strong reactive mothers and had received from their father a +-*(rc)* chromosome *X*, a *Cy-(rc)* chromosome *2* and, for about half of them, a *se-(rc)* chromosome *3*, was measured. The finding of a G_4 infertile female would mean that at least one of the paternally inherited *rc* chromosomes had been contaminated in G_2 females. The fertility of 28 to 35 G_4 females was measured in the progeny of each of the 16 G_1 males.

As a control, the individual fertility of 41 females +-*(r)*/+-*(r)*; *Cy-(r)*/+-*(r)*; *se-(r)/se-(r)* was measured. These females came from a mass cross between 20 seF_5 strong reactive females and 25 G_2 males +-*(r)*; *Cy-(r)*/+-*(i)*; *H-(r)/se-(r)*, taken at random in the progenies of the 16 G_1 males.

The results are given in Table 2. As it might be expected, contamination occurred in G_2 females bearing a +-*(i+)* chromosome *2*. Indeed, in the progenies of the four G_1 males that had been previously detected as bearing a +-*(i+)* chromosome *2*, the majority of G_4 females display strongly reduced fertility and therefore have inherited from their father at least one rc^+ chromosome. In contrast, in the progenies of the 12 G_1 males carrying a +-*(i0)* chromosome *2*, G_4 females are more fertile. The 12 distributions of G_4 females are not homogeneous since the χ^2 is 36.9 for 11 degrees of freedom ($p < 0.001$). Nevertheless, the distribution showing the lowest proportion of females between 80 and 100% (second i^0 column from the left) does not differ from the control distribution ($\chi^2 = 1.15$; d.f = 1; $0.30 > p > 0.20$). Therefore, G_4 females of the 12 distribution are either as fertile or more fertile than the control distribution, but never less fertile. This means that these G_4 females have inherited only noncontami-

TABLE 2

Chromosomal contamination in G_2 females carrying either a +—(i+) or a +—(i0) chromosome 2 as a contaminating element

G_2 female with +—(i) *2* :		i^+	i^+	i^+	i^+	i^0	i^0	i^0	i^0	i^0	i^0	i^0	i^0	i^0	i^0	i^0	i^0	Control
Hatching	0–5%	32	31	29	30	0	0	0	0	0	0	0	0	0	0	0	0	0
percent	5–20%	0	2	3	5	0	0	0	0	0	0	0	0	0	0	0	0	0
of the	20–40%	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
eggs laid	40–60%	0	0	0	0	1	2	0	0	0	1	1	0	0	1	2	0	1
by G_4	60–80%	0	0	0	0	5	15	11	1	12	4	4	2	8	9	9	5	14
females	80–100%	0	1	0	0	26	18	24	29	24	31	27	31	25	18	20	27	26

The Table gives for each progeny of the 16 G_1 males, the distribution of G_4 females according to their fertility. In the upper line is indicated the i^+ or i^0 character of the +—(*i*) chromosome *2* carried by G_2 females. The right-hand column gives the distribution of control females according to their fertility.

nated *rc* chromosomes from their father and consequently that contamination has not occurred in the G_2 females carrying a $+$-*(i°)* chromosome *2*. Therefore, it can be concluded that i^0 chromosomes are not able to act as contaminating elements under conditions in which i^+ chromosomes can do so.

Ability of $+$-(i°) *Luminy chromosomes* 2 *to be contaminated*

In order to ascertain whether i^0 chromosomes can be contaminated by i^+ chromosomes in females in which contamination takes place, an experiment was made with the progenies of seven G_1 males *Basc-(r): Pm-(r)/+-(i°)*; *H-(r)/+-(i+)* taken at random among the 12 G_1 males which had previously been found to carry a $+$-*(i°)* chromosome *2*.

The experimental scheme is given in Figure 2. In each progeny of the seven G_1 males, G_2 males were recovered that, except the $+$-*(i°)* chromosome *2*, carry major chromosomes from reactive origin. Mated to inducer females, they give rise to G_3 females bearing three kinds of major chromosomes: inducer chromosomes inherited from their mother, *X* and *3* reactive originating chromosomes and $+$-*(i°)* chromosome *2* inherited from their father. These females carry the inversions *In(2L+2R)Cy* and *In(3LR)DcxF*, which strongly inhibit recombination on chromosomes *2* and *3*. Since G_3 females result from a cross between

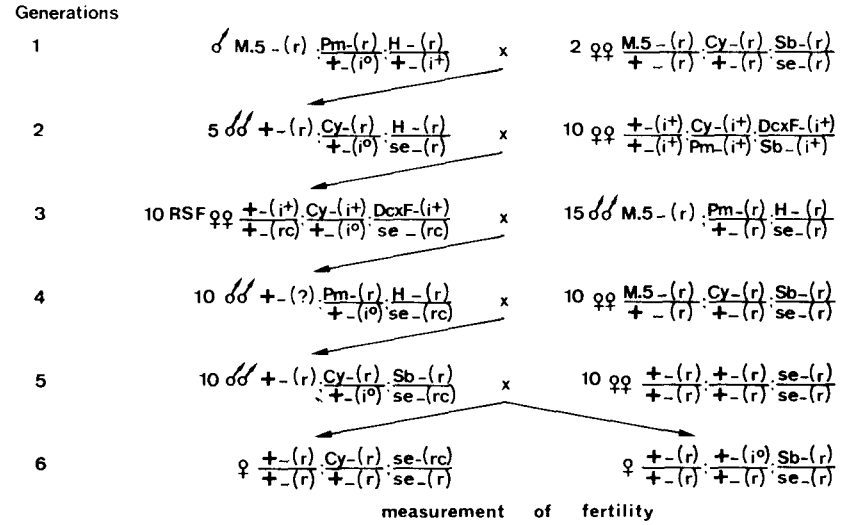

FIGURE 2.—Mating scheme to test the ability of the $+$-*(i°)* chromosomes *2* to be contaminated. The same matings were made starting from seven of the 12 G_1 males bearing an i^0 chromosome *2* (see text). These males come from a mass cross between 40 females *Basc-(r)/M.5-(r)*; *Cy-(r)/Pm-(r)*; *H-(r)/Sb-(r)* of the reactive stock LH_{12} and 60 males of the wild-type Luminy inducer stock. Reactive females used at generations one and four and reactive males used at generation three come from a cross between the LH_{12} and *se*F_5 reactive stocks. Reactive females of generation five are from the *se*F_5 strong reactive stock. Inducer females used at generations two come from crosses between the *Cy/Pm*; *H/Sb-(I)* and the *DcxF/Sb-(I)* stocks. Chromosomes bearing *Cy*[= *In(2L+2R)Cy*] and *DcxF*[= *In(3LR)DcxF*] are used as crossing over inhibitors. (M.5 in the figure = *Basc*, *i.e.*, Muller-5.)

inducer females and males that bear almost only reactive originating chromosomes, they may be considered as *RSF* females; therefore, it may be expected that chromosomal contamination takes place in them. In the following generations, the $+$-*(i°)* chromosomes *2* transmitted from such females were tested for their inducer character. To check the effective occurrence of contamination in G_3 females, the *se-(rc)* chromosomes *3* were also tested. The principle of the test is always the same and consists of measuring the fertility of daughters of strong reactive females and which have inherited from their father either the $+$-*(i°)* chromosome *2* or the *se-(rc)* chromosome *3* and chromosomes of reactive origin that cannot have been contaminated. Such females were produced at generation six, and for each indicated genotype, the individual fertility of about 30 to 35 of them was measured.

The results, given in Figure 3, lead to three observations: (1) In each of the seven progenies, a variable proportion of G_6 females carrying the *se-(rc)* chromosome show a highly reduced fertility. This means that these females bear a *se-(rc⁺)* chromosome and therefore that contamination has occurred in G_3 *RSF* females. (2) The same observation can be made for G_6 females carrying the $+$-*(i°)* Luminy chromosome *2*. Therefore, these chromosomes can be contaminated in *RSF* females. (3) The data do not allow a strict quantitative analysis

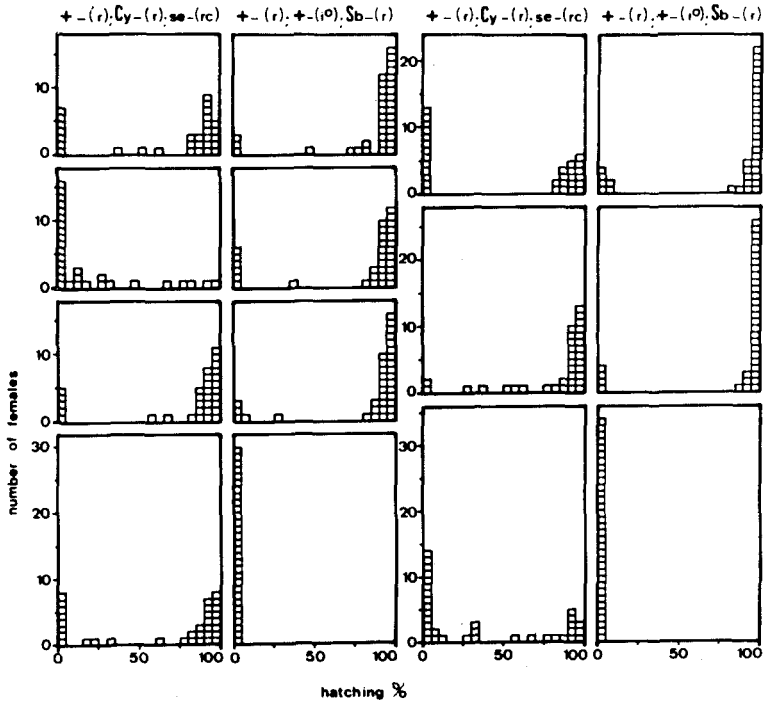

FIGURE 3.—Test of the inducer character of the *i°* chromosome *2* and *se-(rc)* chromosomes *3* after contamination in *RSF* females. Each square represents the fertility of a G_6 female that has inherited from its father the gamete indicated on the top of the columns of histograms. Each line of two histograms corresponds to G_6 females coming from the same G_1 male.

of the frequencies of contamination since the *se-(rc)* and +-*(i°)* chromosomes tested in G_6 females derive after two uncontrolled clonings from ten chromosomes directly transmitted from the *RSF* females. Nevertheless, it may be noticed that +-*(i°)* chromosomes *2* do not appear to be contaminated with a frequency systematically lower than *se-(rc)*.

It can be concluded from this experiment that the +-*(i°)* Luminy chromosomes *2* are similar to chromosomes from reactive origin in their ability to be contaminated.

Segregation of chromosomes 2 +-(i^+) *and* +-(i^o) *in the progeny of Luminy males and females*

An experiment was performed to verify that the three possible genotypes are actually found in the Luminy stock and to study the segregation of +-*(i⁺)* and +-*(i°)* chromosomes *2* in the progeny of heterozygous males and females.

Forty-six males were taken at random in the Luminy stock. Each of them was individually mated with three females *Basc-(r)/Basc-(r)*; *Cy-(r)/Pm-(r)*; *H(r)/Sb-(r)* of the reactive stock LH_{23}. In the progeny of each cross, about 11 G_1 males *Basc-(r)*; +-*(i)/Pm-(r)*; +-*(i⁺)/Sb-(r)* were recovered and individually mated with three *Basc-(r)/+-(r)*; *Cy-(r)/+-(r)*; *H-(r)/se-(r)* females produced by a cross between the reactive stocks seF_5 and LH_{23}. The +-*(i)* chromosome *2* of each of the 46 × 11 G_1 males was then tested for the inducer character. For this purpose, five males +-*(r)*; +-*(i)/Cy-(r)*; *Sb-(r)/se-(r)* were recovered in the progeny of each G_1 male and mated with five seF_5 strong reactive females. The fertility of a set of about 20 G_3 daughters +-*(r)/+-(r)*; +-*(i)/+-(r)*; *se(r)/se-(r)* was measured. If the +-*(i)* chromosome *2* carried by the G_1 male was i^o, these females will show a normal fertility, whereas if it was i^+, they will show reduced fertility. Thus, the protocol allows testing of the inducer character of about 11 +-*(i)* chromosomes *2* directly inherited from a single male of the Luminy stock and therefore permits determination of the genotype of this male.

The same experiment was made for 21 females taken at random from the Luminy stock. The only difference is that the G_1 males carry the +-*(i⁺)* *X* chromosome instead of *Basc-(r)*, but this does not interfere in any way with the test of the +-*(i)* chromosome *2*.

Table 3 gives the results of the fertility measurements of the 725 sets of G_3 females. As it appears in the right hand column, the hatching percentages, except for 27 of them which will be discussed below, are either lower than 5% or higher than 80%. Therefore, it is easy to distinguish the G_1 males carrying a +-*(i⁺)* chromosome *2*, which give G_3 females with highly reduced fertility, from the G_1 males carrying a +-*(i°)* chromosome *2*, which give normally fertile G_3 females. Thus, the genotype of the Luminy G_0 flies can be determined. Indeed, those that have produced both kinds of G_1 males were heterozygous, and it may be concluded that those that have produced only one kind of G_1 males were homozygous. The data concerning the G_0 flies of the same sex and genotype have been pooled. Thus, Table 3 gives the distributions of the sets of G_3 females that

TABLE 3

Segregation of chromosome 2 in the progeny of the 46 Luminy males and 21 Luminy females

Sex and genotype		Males i^+/i^+	Males i^0/i^0	Males i^+/i^0	Females i^+/i^+	Females i^0/i^0	Females i^+/i^0	Total
Number of G_0 flies		14	10	22	7	3	11	67
Hatching percent	0–5%	144	0	132	71	0	70	417
of the eggs	5–20%	4	0	0	9	0	2	15
laid by the sets	20–40%	0	0	2	1	0	0	3
of G_3 females	40–60%	0	0	0	0	0	0	0
	60–80%	0	1	0	0	2	6	9
	80–100%	0	102	99	0	30	50	281

The Table gives the distribution of the sets of G_3 females according to their fertility. The data concerning the sets of G_3 females that derive from the Luminy flies of the same sex and of the same genotype are pooled.

derive from each of the six classes of G_0 flies, and the number of G_0 flies in each class.

Three observations can be made from these results: (1) The three possible genotypes coexist in the Luminy stock and their proportions do not significantly differ in males and in females ($\chi^2 = 0.54$; d.f. $= 2$; $0.70 < p < 0.80$). It must be also noticed that the observed frequencies of the three genotypes in the stock [$+\text{-}(i^+)/+\text{-}(i^+) = 0.313$; $+\text{-}(i^0)/+\text{-}(i^0) = 0.194$; $+\text{-}(i^+)/+\text{-}(i^0) = 0.492$] are in good agreement with Hardy-Weinberg predictions [$+\text{-}(i^+)/+\text{-}(i^+) = 0.312$; $+\text{-}(i^0)/+\text{-}(i^0) = 0.194$; $+\text{-}(i^+)/+\text{-}(i^0) = 0.493$].

(2) The second observation concerns the segregation of chromosome *2* in the progenies of heterozygous G_0 flies. About 11 chromosomes *2* directly transmitted from each heterozygote have been tested and distributed into two classes, $+\text{-}(i^+)$ and $+\text{-}(i^0)$, according to the fertility of the sets of G_3 females. It was assumed, at least as a first approximation, that a fertility lower than 40% corresponds to a $+\text{-}(i^+)$ chromosome, while a fertility higher than 60% corresponds to a $+\text{-}(i^0)$. The 22 distributions obtained from the 22 heterozygous G_0 males are not significantly different ($\chi^2 = 15.2$; d.f. $= 21$; $0.80 < p < 0.90$), and therefore may be pooled. It is true also for the 11 distributions obtained from the 11 heterozygous G_0 females. ($\chi^2 = 12.3$; d.f. $= 10$; $0.20 < p < 0.30$). It must be first noticed that the frequencies of $+\text{-}(i^+)$ and $+\text{-}(i^0)$ chromosomes *2* in the progenies of G_0 heterozygous males are 0.562 and 0.438, respectively. These frequencies are significantly different from the expected 0.500 and 0.500 ($\chi^2 = 5.26$; d.f $= 1$; $0.02 < p < 0.05$). Therefore, there is a slight segregation distortion. An important point is that the segregation of chromosomes *2* inherited from G_0 heterozygous females [72 $+\text{-}(i^+)$ and 56 $+\text{-}(i^0)$] shows the same distortion and does not differ from that observed for G_0 heterozygous males [134 $+\text{-}(i^+)$ and 99 $+\text{-}(i^0)$] since $\chi^2 = 0.06$ for d.f $= 1$ ($p \simeq 0.80$). No further investigation has been carried out on this slight segregation distortion, which at this time remains unexplained. Nevertheless, the similarities between segregation frequencies within the prog-

enies of heterozygous males and females clearly indicate that on i^+ chromosome *2*, *I* factor is located at a single locus, unless the i^o character is systematically associated with one or several inversions. This hypothesis was easily ruled out by studying the polytene chromosomes of heterozygous +-*(i*$^+$*)*/+-*(i*^{o}*)* larvae from crosses between stocks homozygous for the +-*(i*$^+$*)* and +-*(i*^{o}*)* chromosomes *2*, respectively. These stocks were constructed by mating the +-*(r)*; +-*(i)*/ *Cy-(r)*; *Sb-(r)/se-(r)* G_2 males derived from a single homozygous G_0 male by G_2 females of the same genotype from the same progeny. Therefore, it may be assumed, at least at a first approximation, that in the inducer stock Luminy, the genetic element responsible for the character i^+ or i^o of the chromosome *2* is transmitted in a Mendelian way as a single allelic pair.

(3) It has been noted above that of the 725 sets of G_3 females studied, 27 gave hatching percentages higher than 5% but lower than 80%. Among these 27 sets, nine had hatching percentages between 60% and 80%. As was previously noted for the exceptional females of Table 1, it cannot be asserted without further studies that this slight reduction of fertility has anything to do with *SF* sterility. The 18 other sets gave hatching percentages between 5% and 40%. Therefore, among about 20 G_3 females of each of these sets, a variable proportion showed a higher fertility than expected. In one case, this increase of fertility seemed to result from the presence in the females of a +-*(i*$^+$*)* chromosome *2* having a lower efficiency to induce sterility. Indeed, in the progeny of one of the 7 +-*(i*$^+$*)*/ +-*(i*$^+$*)* females, five sets of G_3 females gave hatching percentages lower than 5% and the seven others hatching percentages between 11% and 40%. It may be supposed that this G_0 female was heterozygous for two genetic types of +-*(i*$^+$*)* chromosomes *2* differing in efficiency of inducing sterility, and therefore that several genetic types of +-*(i*$^+$*)* chromosomes *2* coexist in the Luminy stock. The 11 other sets of G_3 females showing a fertility between 5 and 40% are distributed at random in other progenies of homozygous +-*(i*$^+$*)*/+-*(i*$^+$*)* and heterozygous males and females. If the increase of fertility results from the presence in these sets of females carrying a +-*(i*$^+$*)* chromosome *2* showing a lower efficiency to induce sterility—which remains to be demonstrated—this would mean that during the course of the experiment, some +-*(i*$^+$*)* chromosomes *2* have undergone a change towards a lower inductivity.

Distribution of X, 2 *and* 3 i^o *chromosomes in inducer stocks*

Twenty-four inducer stocks were tested for the inducer character of their chromosomes *2* and *3*. Four of them were old laboratory stocks and the 20 others were established from flies caught in the wild at various places in France and in other countries about seven months before the beginning of the experiment. These stocks have been maintained in the laboratory by endogamous crosses involving about 40 flies at each generation.

Thirty males of each inducer stock were mated with 15 females *Basc-(r)/ Basc-(r)*; *Cy-(r)/Pm-(r)*; *Sb-(r)/H-(r)* from the reactive stock LH_{12}. In the progeny of each cross, 15 heterozygous males *Basc-(r)*; *Cy-(r)*/+-*(i)*; *Sb-(r)*/ +-*(i)* were recovered and mated with ten strong reactive seF_5 females. The

individual fertility of ten to 20 daughters of both *Basc-(r)/+-(r)*; *+-(i)/+-(r)*; *Sb-(r)/se-(r)* and *Basc-(r)/+-(r)*; *Cy-(r)/+-(r)*; *+-(i)/se-(r)* genotypes are measured. These measurements of fertility test the i^+ or i^0 character of either their *+-(i)* chromosome *2* or their *+-(i)* chromosome *3*. In addition, since statistically half of the females of this generation carry a chromosome *4* of inducer origin, its influence was tested by measuring the fertility of a set of about 20 *Basc-(r)/+-(r)*; *Cy-(r)/+-(r)*; *Sb-(r)/se-(r)* females.

As a control, the same experiment was performed using the seF_5 reactive stock instead of the original inducer stock. However, in this case, the fertility of a larger number of females has been measured.

Eight of the 24 inducer stocks were also investigated for the i^+ or i^0 character of their *X* chromosome in the following way: 18 females of each inducer stock were mated with 20 *Basc-(r)*; *Cy-(r)/Pm-(r)*; *Sb-(r)/H-(r)* of the reactive stock LH_{12}. In the progeny of each cross, 15 *+-(i)*; *Cy-(r)/+-(i)*; *Sb-(r)/+-(i)* males were recovered and mated with 15 seF_5 strong reactive females. The fertility of ten to 20 *+-(i)/+-(r)*; *Cy-(r)/+-(r)*; *Sb-(r)/se-(r)* daughters was individually measured.

As before, a control experiment was made using the seF_5 reactive stock instead of the original inducer stock.

The results of the fertility measurements are presented in Table 4. It must be first noticed that the control measurements led to somewhat low hatching percentages. This was due to the fact that the females carried two dominant markers and three in the test of chromosome *4*. Taking into account this fact, the data provide information on three main points: (1) Column 4 gives the hatching percentages of the eggs laid by the sets of about 20 females that carry only major chromosomes from reactive origin, but that may be either homozygous *+-(r)/+-(r)* or heterozygous *+-(i)/+-(r)* for chromosome *4*, with an expected frequency of 0.5. At least 16 values are significantly lower than the control value obtained from a set in which all the females are homozygous *+-(r)/+-(r)* for chromosome *4*. Without further studies, it is not possible to decide whether the reduction of fertility in these 16 sets has anything to do with *SF* sterility and therefore whether it reflects the presence in some females of these sets of a *+-(i^+)* chromosome *4*. Nevertheless, it must be noted that PELISSON (1977) has clearly demonstrated that the Nagasaki strain, which, in this experiment gives the lowest value, is homozygous for a *+-(i^+)* chromosome *4*. Therefore, it may be supposed that in addition to Nagasaki, several other strains carry a *+-(i^+)* chromosome *4*.

(2) The second point which appears from the data is that any major chromosome of inducer strains may be i^0. Indeed, it is clear that the Leningrad and Otanu strains carry i^0 *X* chromosomes, Kerbiniou, Menetreol-B and Leningrad i^0 second chromosomes and Le Montet and Tulle i^0 third chromosomes. It can be concluded that the presence of major i^0 chromosomes in inducer strains is not an exceptional event, since among the 24 tested strains at least six carry them. Moreover, the number of strains carrying major i^0 chromosomes is probably underestimated by the data. The low number of chromosomes tested in each

TABLE 4

Distribution of X, 2 *and* 3 i^0 *chromosomes in inducer stocks*

Strains	4	3						2						X					
		0 5%	5 20%	20 40%	40 60%	60 80%	80 100%	0 5%	5 20%	20 40%	40 60%	60 80%	80 100%	0 5%	5 20%	20 40%	40 60%	60 80%	80 100%
Control	69.3 ± 0.8	0	0	0	1	16	17	0	0	0	0	2	47	0	0	0	1	13	39
Beaune	59.7 ± 4.2	18	0	2	0	0	0	19	0	0	0	0	0						
Bellegarde	69.4 ± 3.8	17	0	0	0	0	0	18	0	0	0	0	0						
Biziat	83.9 ± 2.5	16	0	0	0	0	0	19	0	0	0	0	0						
Bordeaux	59.7 ± 3.6	13	0	0	0	0	0	17	0	0	0	0	0						
Kerbiniou	58.6 ± 1.9	15	0	0	0	0	0	9	2	0	0	1	1	17	0	0	0	0	0
Le Montet	56.3 ± 4.9	4	0	0	2	5	2	17	0	0	0	0	0						
Menetreol A	67.1 ± 4.0	16	0	0	0	0	0	12	0	1	2	1	0						
Menetreol B	61.6 ± 2.5	12	0	0	0	0	0	6	0	0	2	4	4						
Menetreol C	38.9 ± 3.3	18	0	0	0	0	0	13	0	0	0	0	0						
Montluçon A	52.5 ± 3.5	12	0	0	0	0	0	15	0	0	0	0	0						
Montluçon B	50.0 ± 4.4	18	0	0	0	0	0	17	0	0	0	0	0						
Moulins	66.4 ± 3.9	18	0	0	0	0	0	15	0	0	0	0	0						
Nevers	58.5 ± 5.1	4	4	3	1	0	0	9	2	6	1	2	0						
Toulon	57.2 ± 1.8	14	0	0	0	0	0	13	0	0	0	0	0	18	0	0	0	0	0
Tulle	53.1 ± 4.4	3	1	0	0	2	6	13	0	0	0	0	0						
St-Denis	50.2 ± 3.4	17	0	0	0	0	0	18	0	0	0	0	0						
St-Paul	54.9 ± 2.2	15	0	0	0	0	0	17	0	0	0	0	0	8	3	6	1	0	0
Alger	56.8 ± 2.8	16	0	0	0	0	0	14	0	0	0	0	0	15	0	0	0	0	0
Sinferopol	55.5 ± 4.0	13	0	0	0	0	0	14	0	0	0	0	0						
Yalta	58.8 ± 3.9	18	0	0	0	0	0	17	0	0	0	0	0						
Champetières	49.5 ± 2.0	17	0	0	0	0	0	15	0	0	0	0	0	11	3	0	0	1	0

Leningrad	58.8 ± 2.5	3	6	2	0	0	0	5	0	1	3	1	1	0	0	0	5	8	1
Otanu	56.7 ± 2.3	3	4	5	0	0	0	16	0	0	0	0	0	0	0	3	3	7	4
Nagasaki	30.2 ± 1.8	17	0	0	0	0	0	15	0	0	0	0	0	18	0	0	0	0	0

Except Champetières, Leningrad, Otanu and Nagasaki, which have been grown in laboratory for many years, all the strains derive from flies caught recently in the wild. Sinferopol and Yalta come from U.S.S.R., Alger from Algeria, St-Denis and St-Paul from Reunion and the others from France. The letters A, B and C indicate strains derived from flies caught at the same place but at different times. Column 4, gives the hatching percentages of the eggs laid by a set of 20 females *Basc-(r)+—(r)* ; *Cy-(r)/+—(r)* ; *Sb-(r)/se-(r)* among which statistically half of them carry a *+—(i)* chromosome *4*. These values are given with the standard error. In column 3 are the results of the test of *+—(i)* chromosome *3*. Each number is the number of *Basc-(r)/+—(r)* ; *Cy-(r)/+—(r)* ; *+—(i)/se-(r)* females which give individual hatching percentages between the corresponding values indicated at the top of the column. In the same way, columns 2 and X give the results of the tests of *+—(i)* chromosomes *2* and *X*. They give, respectively, the distributions of *Basc-(r)/+—(r)* ; *+—(i)/+—(r)* ; *Sb-(r)/se-(r)* females and *+—(i)/+—(r)* ; *Cy-(r)/+—(r)* ; *Sb-(r)/se-(r)* females according to their fertility.

strain makes it possible to take only +-(i^+) chromosomes in a strain showing a polymorphism with a low frequency of +-(i^o) chromosomes. Also, several strains seems to carry +-(i^+) chromosomes *4*, and females bearing such chromosomes will be poorly fertile even when they carry a +-(i^o) major chromosome. For the same reasons, the data do not allow determination of whether the six strains carrying a +-(i^o) major chromosome display a polymorphism [+-(i^+); +-(i^o)] for this chromosome or are homozygous +-(i^o).

(3) In some stocks several females bearing a +-(*i*) major chromosome laid eggs with hatching percentages between 5% and 60%. Their +-(*i*) chromosome cannot be considered as an i^o chromosome since it induces a reduction of fertility, but this reduction is lower than that induced by the majority of the +-(i^+) chromosomes. This observation supports the hypothesis previously put forward that +-(i^+) chromosomes may differ in their efficiency to induce *SF* sterility.

DISCUSSION

The experiments reported in this paper clearly show that the Luminy inducer stock displays a polymorphism for two kinds of second chromosomes: +-(i^+) chromosomes, which produce *SF* females when they are introduced by a paternal gamete into reactive oocytes and +-(i^o) chromosomes, which in the same conditions produce normally fertile females. In the progenies of heterozygous males, the observed segregation is about 56% +-(i^+) chromosomes to 44% +-(i^o). The cause of this slight distortion has not been investigated, but the most important fact is that heterozygous females, in which genetic recombination takes place, give the same segregation ratio. Therefore, on the +-(i^+) chromosome *2*, *I* factor appears to be located at a single locus and, at least as a first approximation, it may be concluded that, in the Luminy stock, the genetic determinants of the characters i^+ and i^o are inherited as a single allelic pair.

Nevertheless, some observations suggest a somewhat more complex situation. Indeed, in the Luminy stock, a female that seems to segregate two kinds of +-(i^+) chromosomes *2* differing in their efficiency to induce *SF* sterility has been detected. This would mean that several genetic types of +-(i^+) chromosomes may exist. Evidence for this hypothesis was given recently by PELISSON (1978), who demonstrated the coexistence of at least three types of *X* chromosomes in the Otanu inducer strain: i^o chromosomes, and two types of i^+ chromosomes, one of them showing a lower efficiency to induce sterility than the other. Moreover, there is some doubt about the stability of the +-(i^+) and perhaps also of the +-(i^o) chromosomes *2* of the Luminy stock. The experiments reported in this paper do not exclude the possibility that i^o chromosomes induce, in exceptional cases, a partial reduction of fertility and the hypothesis that i^+ chromosomes generate copies showing a lower efficiency to induce *SF* sterility cannot be ruled out. These points are presently being investigated in our laboratory.

The survey of four old laboratory inducer strains and of 20 strains coming from flies caught in the wild about seven months ago indicates that at least six of them carry i^o chromosomes and that any chromosome of an inducer strain

may be i^o. These results are in agreement with those reported by KEARSEY *et al.* (1977), who found, in laboratory strains maintained in population cage, a polymorphism for chromosomes *2* and *3* capable of inducing female sterility. Nevertheless, the physiological characteristics of this sterility have not been investigated and therefore its identity with *SF* sterility, although highly probable, is not demonstrated. It must be noted, however, that at present, the available data do not permit any decision as to whether i^o chromosomes exist in the wild or whether they appear only when flies are grown in laboratory conditions. Whatever the case, the existence of *X*, *2*, *3* and *4* i^o chromosomes makes it possible to construct a stock bearing only i^o chromosomes. The construction of this stock is being carried out in our laboratory and the study of its behavior should be of great interest.

At least regarding *I* factor inheritance, the $+$-(i^o) second chromosomes of the Luminy strain behave like chromosomes originating from reactive strains. Indeed, as *r* chromosomes, i^o chromosomes are unable either to induce *SF* sterility or to act as contaminating elements, and they may be contaminated as frequently as *r* chromosomes when they are in *RSF* females (and therefore probably also in *SF* females) with i^+ chromosomes. Nevertheless, it cannot be accepted without further investigation that i^o chromosomes are entirely similar to *r* chromosomes. It was shown (BUCHETON and PICARD 1978; PICARD 1978a,b) that *r* chromosomes are necessary to maintain the cytoplasm of reactive strains in a reactive state, whereas the introduction of i^+ chromosomes brings about the loss of the reactive character of the cytoplasm. There are at present no data concerning the effects of i^o chromosomes on the maintenance of the reactive condition. Perhaps the stock carrying only i^o chromosomes will permit us to answer this question.

The most important conclusion to be drawn from the observations made on the Luminy $+$-(i^+) chromosomes *2* is that chromosomal contamination with its characteristic feature, high frequency of the transformation and restriction to females, does not take place in the Luminy stock. Indeed, in spite of their ability to be contaminated by i^+ chromosomes in *RSF* females, they are maintained in the Luminy strain with i^+ chromosomes. Moreover, in the progenies of heterozygous $+$-$(i^+)/+$-(i^o) Luminy females, the segregation of chromosome *2* is the same as that observed for heterozygous males. It can be concluded, therefore, that chromosomal contamination does not take place in Luminy females. Since the ability to be contaminated in *SF* and *RSF* females has been observed for other i^o chromosomes, the finding of i^o chromosomes in several inducer strains means that, more generally, chromosomal contamination does not take place in inducer females and therefore that its occurrence requires a cytoplasm in a reactive state. This conclusion agrees with the results previously reported (PICARD 1978c) showing that in *SF* females the frequency of contamination is directly correlated with the "level of reactivity" (which varies from strong to weak) of their reactive mother. The occurrence of contamination in *RSF* females may, at first sight, appear to be in contradiction to this conclusion, since they are produced by inducer mothers and therefore receive an inducer originating cytoplasm. Nevertheless, it was previously demonstrated (PICARD 1978a,b) that some

daughters of *RSF* females show variably reduced fertility and therefore must have received a cytoplasm in a reactive state. Since *RSF* females can produce oocytes with a cytoplasm in reactive state, it may be assumed that at least some of their germ cells carry a reactive cytoplasm that allows contamination to occur.

The existence of i^0 chromosomes and the lack of chromosomal contamination in inducer females open an interesting way for mapping *I* factor on i^+ chromosomes. Several assays have been made in our laboratory and the results will be published in a later paper.

Finally, it may be noticed that the results presented in this paper strengthen the analogy pointed out in the preceding paper (PICARD 1979) between *I* factor and the genetic element(s) responsible for male recombination, which is one of the most investigated traits of hybrid dysgenesis. Like i^+ chromosomes, chromosomes able to induce male recombination are found in natural populations in various geographical locations. Both chromosomes *2* and *3* may exhibit this characteristic and some populations display a polymorphism (HIRAIZUMI 1971; VOELKER 1974; WADDLE and OSTER 1974; KIDWELL and KIDWELL 1975, 1976; SVED 1976; WOODRUFF and THOMPSON 1977). Moreover, when SLATKO and HIRAIZUMI (1975) tried to map the genetic element(s) on the second chromosome of line T007, they obtained complex results that led them to invoke one "major element" and "evanescent secondary elements." These data fit well both with the observation that on the i^+ Luminy chromosome *2*, *I* factor is located at one site and with chromosomal contamination.*

However, this analogy is in no way sufficient evidence for concluding that *SF* sterility and male recombination are associated. Indeed, preliminary experiments carried out in our laboratory failed to demonstrate a systematic association between these two syndromes. A question raised by this analogy is whether the chromosomal elements responsible for male recombination have the same genetic behavior as *I* factor. It is impossible at this time to provide an answer since no detailed genetic analysis of these elements is directly comparable with that of *I* factor and since the genetic determinism of the maternal component with which they interact is unknown.

We are grateful to J. M. LAVIGE for observations of salivary glands chromosomes and to J. C. BREGLIANO and P. L'HERITIER for advice throughout this work and for helpful comments on the manuscript. This work was suported by grants from the C.N.R.S. (E.R.A. 692), the University of Clermont-Ferrand II and the Fondation pour la Recherche Médicale Française.

* *Note added in proof*: The analogy between *I* and *Mr* factors is strengthened by recent data from SLATKO (1978, Genetics **90**: 105–124), who reported a chromosomal contamination-like phenomenon for the *Mr* factor.

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