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Non-mendelian female sterility and hybrid dysgenesis in *Drosophila melanogaster*

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SUMMARY

Systematic crosses between various strains of *Drosophila melanogaster* lead in some cases to partly sterile F_1 females (*SF* females). Two main classes of strain, inducer and reactive, have been defined on the basis of this sterility, which shows very specific physiological features. *SF* females arise only when reactive females are crossed with inducer males. In contrast, F_1 females (*RSF*) produced by the reciprocal cross between inducer females and reactive males have normal fertility. All wild populations tested are of the inducer category, laboratory strains are either inducer or reactive. Sterility is the result of interaction between two genetic factors denoted *I* and *R*, respectively responsible for the inducer and reactive conditions and whose unusual genetic behaviour has been described in other papers. The present paper reports experiments showing that the *I–R* interaction is also responsible for high levels of *X* nondisjunction and of mutation in the *SF* female germ-line. The analogy with the P-M system of Kidwell, Kidwell & Sved (1977*b*), is discussed as are also the implications of the existence of the *I-R* system for spontaneous mutation research in *D. melanogaster*.

1. INTRODUCTION

It was shown previously (Picard, 1971) that in some cases crosses between various strains of *Drosophila melanogaster* produce F_1 females which exhibit a specific kind of sterility, resulting from the failure of some eggs to complete embryonic development. This phenomenon, called *SF* sterility, exhibits the following characteristic features which allow it to be distinguished from any other kind of female sterility in *Drosophila* (Picard *et al.* 1977). (i) Unhatched eggs are fertilized but they do not reach the blastoderm stage. Hatched eggs produce flies which do not show any conspicuous developmental abnormality. (ii) The hatching percentage always rises as F_1 females age. (iii) Hatchability rises quickly but reversibly when egg-laying *SF* females are put at 30 °C. (iv) The probability

of embryonic death does not depend on genotype, the mates of *SF* females do not intervene.

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 crosses involving a neutral strain give only fertile F_1 females. The reduced fertility appears only in F_1 daughters (denoted *SF* females) from a cross between reactive females and inducer males. Reciprocal crosses lead to normally fertile females (denoted *RSF* females), but reduced fertility may appear in their daughters (Picard, 1978*a*, *b*). A survey of about 200 strains has indicated that only the inducer condition is found in the wild, whereas strains belonging to the three classes are maintained in several European and American laboratories (Picard *et al.* 1976, and unpublished results).

The inducer and reactive conditions show a large quantitative variability; according to the choice of both reactive and inducer parental strains, the hatching percentage of the eggs of young *SF* females varies within broad limits at 20 °C (Bucheton *et al.* 1976). In reactive strains, 'levels of reactivity' have been defined which can be measured by the fertility of the *SF* females produced by crosses with a standard inducer strain. Thus, *strong* reactive females or *weak* reactive females can be distinguished, and also intermediate types. A similar variability is found among inducer strains. Several lines of evidence indicate that neutral strains may be considered as extremely *weak* reactive strains, unable to give any noticeable *SF* sterility (Picard, 1978*c*).

It has been demonstrated that *SF* sterility results from an interaction between two genetic factors, denoted *I* and *R*, respectively brought to the F_1 females by the inducer father and the reactive mother. *I* is a chromosomal factor which may be linked to any of the three major chromosomes of inducer strains. In males its transmission flows a mendelian pattern, but in *SF* and *RSF* females, chromosomes of reactive origin may acquire the *I* factor with a high frequency. This phenomenon, which occurs independently of the production of recombined gametes, has been called chromosomal contamination (Picard, 1976). It has been proved that, as for female sterility, this contamination results from an interaction between *I* and *R* factors. In various inducer strains a polymorphism exists between chromosomes bearing the *I* factor (denoted i^+) and chromosomes lacking the *I* factor (denoted i^0). This situation has made possible the building of a stock containing exclusively i° chromosomes (A. Pelisson, in preparation).

The hereditary transmission of the *R* factor, responsible for the reactive state, exhibits complex patterns belonging to both chromosomal and non-chromosomal inheritance. The essential features are summarized as follows. Reactivity is a cytoplasmic state, controlled by the genotype; each genotypic modification determines a change in the level of reactivity but the transition towards the new cytoplasmic state corresponding to the new genotype is always very slowly effected (Bucheton, 1973; Bucheton & Picard, 1975, 1978).

Non-genetic agents such as thermic treatment and age of females can modify the level of reactivity and this modification is partly heritable (Bucheton, in

preparation). The genetic behaviour of the reactivity exhibits striking similarities with the extrachromosomal element *delta* (Minamori, 1969, 1972) but a common basis for the two conditions seems excluded.

A similar kind of nucleocytoplasmic interaction has been described (Kidwell & Kidwell, 1975; Kidwell, Kidwell & Ives, 1977*a*; Kidwell *et al.* 1977*b*). These authors reported that male and female sterilities, associated with several dysgenic traits, occur in the F_1 from crosses between various strains. On the basis of this 'hybrid dysgenesis', Kidwell, Kidwell & Sved classified their strains in two groups called *P* and *M*. There appear to be many similarities but also some clear differences between the two systems. The results of a series of crosses between representatives of *I* and *R* strains on the one hand and *P* and *M* strains on the other strongly suggest that the *I–R* and *P–M* systems are causally independent (M. G. Kidwell, in preparation).

The purpose of this paper is to investigate the occurrence of dysgenic traits, particularly *X* chromosome non-disjunction and visible mutations, in the *I–R* system. The results being positive, their implications for studies of so-called 'mutator systems' in *Drosophila* are discussed in the light of our knowledge about *I* and *R* genetic behaviour.

2. MATERIALS AND METHODS

Genetic symbols are those used by Lindsley & Grell (1968). Flies were grown on the axenic food described by David (1959), at 20 °C. All crosses were made by mass matings.

(i) *Stocks of* Drosophila melanogaster

(*a*) *Reactive.* e_{st} is an original *strong* reactive strain, homozygous for the *ebony* mutation. seF_8 is a *strong* reactive stock, established by selection following the method described in Picard *et al.* (1972), starting from the original *se* reactive strain, homozygous for the *sepia* mutation. $H.J._{30}$ is a *weak* reactive stock selected from the original wild-type *Hikon J.* strain. *v* is an original *weak* reactive stock, homozygous for the *vermilion* mutation.

(*b*) *Inducer. Luminy* and *B2'* are wild-type stocks bred from a small number of flies caught in the wild some years ago respectively in Southern and Western France. *w ct f* and *ese-(I)* are laboratory stocks, respectively homozygous for the mutations *white, cut, forked* and *ebony, sepia.*

(ii) *Measurements of fertility*

Two or three days after emergence about 20 mated females were put in a culture vial containing food stained with carbon black. A sample of about 200 eggs was collected on this food during a period of 24 h. The eggs, which were easily seen on the black background, were recorded as hatched or not hatched 48 h later. Females were mated with their brothers since it has been shown that the hatching percentage of eggs does not depend on the male used to fertilize *SF* females (Picard *et al.* 1977).

3. RESULTS

(i) *Non-disjunction of* X *chromosomes in* SF *females*

Several experiments were performed to measure the frequencies of *X* chromosome non-disjunction in *SF* and *RSF* females coming from various crosses and in F_1 females resulting from crosses between different inducer or between different reactive strains. These experiments were numbered from 0 to 10 and except exp. 0, all were made following the same general scheme.

Exp. 0 was carried out using *SF* females required for another experiment which bears no relation with the matter of this paper. These females had a genotype *w ct f*(i^+)/+(*r*); *Cy*(*r*)/+(*r*); *DcxF*(*r*)/*se*(*r*). They were the progeny of *seF*$_8$*strong* reactive mothers and *w ct f*(i^+); *Cy*(*r*)/+(i^+); *DcxF*(*r*)/+(i^+) fathers coming from appropriate crosses between the *w ct f* inducer stock and two reactive stocks carrying dominant markers. A hundred *SF* females were mated with males of the stock *v*, homozygous for the *vermilion* *X*-linked mutation. They were placed on fresh food each day and the production of successive days was tested. The non-disjunction of *X* chromosomes in *SF* females was measured by scoring the *XO* males in their offspring. These males, which had not received any *X* chromosome from the mother, but which had inherited the *X* chromosome of the father, displayed a *vermilion* phenotype. It was verified that all these males were sterile, as expected for *XO* males. For this purpose, each of them was mated with three *Luminy* females.

In Expt 1 two independent mass crosses were made, each involving 15 *seF*$_8$ *strong* reactive females and 20 *w ct f* inducer males. In the progeny of each cross, 5 sets of 10 *SF* females were recovered and mated each with 15 males of the *v* stock. As previously, the eggs produced on successive days were allowed to develop and the *XO vermilion* males scored in the progeny of each of the 10 sets of *SF* females. The same experiment, denoted 1R, was performed starting from the original reciprocal cross, between *w ct f* females and *seF*$_8$ males, which gives rise to *RSF* females.

Expts 2 and 2R were made in the same way from the two reciprocal crosses between the *seF*$_8$ *strong* reactive and *Luminy* inducer stocks. However, in this case and in all the following experiments, F_1 females were mated with males of the *w ct f* stock and *X* non-disjunction was measured by scoring the *XO* male progeny displaying a *w ct f* phenotype.

Expts 3 and 3R were made from parental reciprocal crosses between the *H.J.*$_{30}$ *weak* reactive stock and the *Luminy* inducer stock.

In Expts 4 and 4R the parental stocks used were the *e*$_{st}$ *strong* reactive and the *Luminy* inducer stocks. Furthermore, in each experiment 4 independent original mass crosses were made instead of 2, and therefore 20 sets of 10 *SF* or *RSF* females were studied instead of 10.

Expts 5 and 6 were control experiments each involving parental crosses between two inducer stocks. In Expt 5 crosses were made between the *B2′* and *Luminy* stocks, and in Expt 6 reciprocal crosses were made between the *ese*-(*I*) and *Luminy* stocks.

Expts 7 and 8 were control experiments starting from parental crosses between reactive stocks. Expt 7 involved reciprocal crosses between the *strong* reactive stocks e_{st} and seF_8 and Expt 8 reciprocal crosses between the *strong* reactive stock seF_8 and the *weak* reactive stock $H.J._{30}$.

In the last two experiments, 9 and 10, the crosses giving rise to *SF* females involved *Luminy* inducer males and heterozygous reactive females produced from the two original crosses of Expt 8 between the *strong* reactive stock seF_8 and the *weak* reactive stock $H.J._{30}$. These reactive females had the same genotype, but in Expt 9 they came from seF_8*strong* reactive females while in Expt 10 they came from $H.J._{30}$ *weak* reactive females. Since it is known (Bucheton, 1973; Bucheton & Picard, 1978) that in crosses between reactive strains the level of reactivity is mainly maternally inherited, it was expected that the $seF_8/H.J._{30}$ reactive females of Expt 9 would have a *stronger* level of reactivity than those of Expt 10 and therefore that *SF* females of Expt 9 would be less fertile than those of Expt 10.

In each experiment the fertility of 4 sets of about 20 *SF*, *RSF* or F_1 control females was measured.

The results are given in the left part of Table 1. They clearly show a strong correlation between the fertility of F_1 females and the frequency of *XO* males in their first 10 days progeny. In the progenies of control F_1 females produced from crosses between inducer stocks (Expts 5 and 6) and between reactive stocks (Expts 7 and 8) this frequency remains between 10^{-3} and 10^{-4}. Frequencies of *XO* males of the same order of magnitude are observed in the progenies of *RSF* females (Expts 1R, 2R, 3R and 4R) and of *SF* females of Expt 3 which come from *weak* reactive mothers and show only a very weak reduction of fertility. In contrast, *SF* females showing a strong reduction of fertility (Expts 0, 1, 2, 4 and 9) produced more than 10^{-2} *XO* males among the surviving offspring from their first 10 days of laying.

Moreover, it should be noted that the frequency of *XO* males is significantly higher in the progenies of $seF_8/H.J._{30}$ *SF* females (Expt 9) which exhibit a strongly reduced fertility, than in the progenies of $H.J._{30}/seF_8$ *SF* females (Expt 10) which are more fertile ($\chi^2 = 21$; D.F. $= 1$; $P < 0{\cdot}001$). However, these *SF* females bear the same genotype. Therefore the frequency of *XO* males in the offspring of *SF* females depends, as for their fertility, on the reactivity level of the cytoplasm inherited from the reactive mothers. It may be concluded that *SF* sterility and a high rate of *X* chromosome non-disjunction are closely correlated and result from the interaction between the same genetic factors.

The data presented in Table 2 strengthens this conclusion. They concern only the experiments in which *SF* females show a strongly reduced fertility in their first days of laying (see Table 1), and clearly indicate that the frequency of *XO* males in the progenies of *SF* females decreases with ageing of these females. Since one of the more specific characteristics of *SF* sterility is the decrease of the probability of embryo death with the ageing of *SF* females (Picard *et al.* 1977) this observation corroborates the existence of a close correlation between *SF* sterility and high rate of *X* chromosomes non-disjunction.

Table 1. *Female fertility*, X *non-disjunction and number of visible mutations from various crosses*

Expt no.	Cross	♂♂ mates of F_1 ♀♀	Hatching (% of eggs laid by F_1 ♀♀ ± S.E.)	*XO* ♂♂/ tot. ♂♂*	% *XO* ♂♂ ± S.E.	Minimum number of mutations scored in ♂♂ Sure	♂♂ Prob.	♀♀ Sure	♀♀ Prob.	Total G2 flies
0	♀♀ seF_8 × ♂♂ heteroz.	*v*	0·1	89/2912	3·05 ± 0·31	Not checked				
1	♀♀ seF_8 × ♂♂ *w ct f*	*v*	4·1 ± 0·8	11/345	3·19 ± 0·95	2	0	1	0	7645
1R	♀♀ *w ct f* × ♂♂ seF_8	*v*	92·4 ± 1·1	0/5455	0·00	0	0	0	0	10911
2	♀♀ seF_8 × ♂♂ *Luminy*	*w ct f*	0·0	12/204	5·88 ± 1·65	4	2	0	1	4543
2R	♀♀ *Luminy* × ♂♂ seF_8	*w ct f*	87·4 ± 1·2	2/3321	0·06	0	0	0	0	6642
3	♀♀ $H.J._{30}$ × ♂♂ *Luminy*	*w ct f*	83·1 ± 1·2	4/6435	0·06	3	0	2	0	19040
3R	♀♀ *Luminy* × ♂♂ $H.J._{30}$	*w ct f*	97·7 ± 0·5	0/6012	0·00	0	0	0	0	13620
4	♀♀ e_{st} × ♂♂ *Luminy*	*w ct f*	0·0	32/1402	2·28 ± 0·41	3	0	6	2	11003
4R	♀♀ *Luminy* × ♂♂ e_{st}	*w ct f*	95·6 ± 0·5	5/12685	0·04	1	1	2	0	31288
5	♀♀ *B.2′* × ♂♂ *Luminy*	*w ct f*	98·1 ± 0·5	2/4001	0·05	0	0	0	0	8003
	♀♀ *Luminy* × ♂♂ *B.2′*	*w ct f*	97·6 ± 0·6	1/4333	0·02	0	0	0	0	8666
6	♀♀ *e-se-(I)* × ♂♂ *Luminy*	*w ct f*	81·6 ± 1·3	2/6255	0·03	0	0	1	0	12511
	♀♀ *Luminy* × ♂♂ *ese-(I)*	*w ct f*	82·0 ± 1·3	1/5706	0·02	0	0	0	0	11411
7	♀♀ e_{st} × ♂♂ seF_8	*w ct f*	79·2 ± 1·6	0/4267	0·00	0	0	0	0	8535
	♀♀ seF_8 × ♂♂ e_{st}	*w ct f*	80·0 ± 1·6	3/3659	0·08	0	0	0	0	7319
8	♀♀ seF_8 × ♂♂ $H.J._{30}$	*w ct f*	98·3 ± 0·5	1/4840	0·02	0	0	0	0	9681
	♀♀ $H.J._{30}$ × ♂♂ seF_8	*w ct f*	98·4 ± 0·5	0/5455	0·00	0	0	0	0	10911
9	♀♀ $seF_8/H.J._{30}$ × ♂♂ *Luminy*	*w ct f*	0·0	11/413	2·66 ± 0·79	0	0	2	0	3951
10	♀♀ $H.J._{30}/seF_8$ × ♂♂ *Luminy*	*w ct f*	51·2 ± 1·4	26/4443	0·58 ± 0·11	1	0	1	1	8887

* The total number of males given in the fifth column includes only those recovered from the first 10 days of laying. It is an estimated number because only the total number of flies in each progeny was counted, without distinguishing between males and females. This estimation is possible since it has been shown that SF sterility does not induce any appreciable sex-ratio modification (Picard *et al.* 1977).

Table 2. *Percentages of* XO *males* ± S.E. *in the male offspring from successive days of laying by* SF *females*

Expt no.	Age of *SF* females in days after emergence (days) 0–5	5–10	10–15	15–20	20–25
0	4·62 ± 0·75	2·51 ± 0·34	0·63 ± 0·13	0·39 ± 0·11	0·28 ± 0·08
1	No progeny	3·19 ± 0·95	2·49 ± 0·66	1·23 ± 0·31	0·89 ± 0·23
2	No progeny	5·88 ± 1·65	4·05 ± 0·86	1·79 ± 0·43	2·00 ± 0·57
4	2·97 ± 1·61	2·23 ± 0·11	2·19 ± 0·36	1·14 ± 0·21	
9	2·04 ± 2·02	2·75 ± 0·86	2·53 ± 0·62	1·29 ± 0·37	

(ii) *Mutability in SF females*

Except for Expt. 0, F_1 female progenies were also checked for visible mutations. To facilitate the search for flies displaying abnormal phenotypes, only eyes, bristles, body colour and size and shape of the wings were examined. It should be noted that in all experiments the protocol allows detection of recessive mutations in males only when they are *X* linked but are not lethal. In females, recessive mutations can be detected only when they are allelic with the mutations carried by the paternal *X* chromosome. In other words, recessive mutations can be detected in females only at the *vermilion* locus in Expts 1 and 1R and only at the *white*, *cut* and *forked* loci in all other experiments. Non-lethal dominant mutations on any chromosome can be detected, of course, in all experiments, in both males and females.

Each fly showing an abnormal phenotype was recovered and appropriate crosses were made in order to determine whether the phenotypic change was inheritable, *i.e.* whether it resulted from a mutation. Each mutation which was verified in this way was denoted a 'sure' mutation. However, some of the flies displaying an abnormal phenotype were prematurely dead or sterile and therefore the genetic basis of the phenotypic change could not be tested. These flies were assumed to carry a 'probable' mutation when the phenotype they displayed was similar to a well-known mutation.

The results are given in the right part of Table 1. Among the 60 370 flies examined in the progenies of F_1 control females (Expts 5–8) only 1 carried a new mutation. In contrast, among the 27142 flies coming from *SF* females which showed a strong reduction of fertility at the beginning of their life (Expts 1, 2, 4 and 9), 18 new mutations have been detected with certainty and 5 others are probable. Seven sure mutations and one probable appear also among the 27927 progeny of *SF* females which display only a weak reduction of fertility (Expts 3 and 10). No mutations have been found in the progenies of *RSF* females, except in Expt 4R. Even in this case, the proportion of visible mutations is significantly lower than in the *SF* females of Expt 4 which come from the reciprocal cross ($\chi^2 = 15{\cdot}0$; D.F. $= 1$; $P < 0{\cdot}001$). Therefore, in *SF* females and, to a lesser degree, in some *RSF* females, mutations arise with an unusually high rate.

Among the 14 sure mutations scored in males, only 1 was dominant. It is located

on the third chromosome and gives a phenotype similar to that observed for *Stubble*. The 13 others are *X*-linked recessive mutations. Three are alleles of *singed*, 3 are at the *yellow* locus and 3 are at the *white* locus, but give brown-coloured eyes. The 4 remaining have not yet been identified: 2 lead to brown-coloured eyes but are not allelic with *white*, and 2 to *beadex*-like phenotype. The 3 probable mutations give respectively apricot, brown-coloured eyes and yellow body. All the 15 sure mutations scored in females are *X* linked, 3 are dominant and give a *beadex*-like phenotype. Among the 12 recessive mutations, 7 are alleles of *cut*, and 5 are alleles of *white* (4 giving white eyes and one giving dark red eyes). No *forked* or *vermilion* mutation has been detected. Three probable mutations give white eyes and the other a *Stubble*-like phenotype.

Eight of these 36 mutations appeared in clusters, suggesting premeiotic mutational events. The cluster size was always small and varied from 2 to 4 flies. Each of the 28 other mutations was scored in a single fly.

Another important point to be noted is that among the 15 sure mutations detected in females, which are all *X*-linked mutations, 14 were found to be associated with a recessive lethal mutation. For 4 of them salivary gland chromosomes were observed, and in 1 case a deletion was visible. Lastly, it may be noted that, although not systematically checked, 6 females displaying a *vermilion* phenotype were found among the 25063 progeny of *SF* females in Expt 0. Five of these females were entirely sterile and the other fertile. It was demonstrated that the *vermilion* mutation carried by this female is associated with a recessive lethal mutation and the observation of salivary gland chromosomes allowed detection of a deletion in the *vermilion* region. Therefore, mutations arising in *SF* females seem to be small deletions rather than point mutations.

4. CONCLUSIONS AND DISCUSSION

The experiments reported in this paper clearly demonstrate that *SF* sterility and a high level of *X* chromosome non-disjunction are strongly correlated. High frequencies of *X* non-disjunction were observed in *SF* females showing a *strong* reduction of fertility but not in fertile *RSF* females nor in inducer and reactive control females. Furthermore, as for the hatching percentage of the eggs laid by *SF* females, the frequency of *X*-chromosome non-disjunction in these females depends on the level of reactivity of the cytoplasm they have inherited and decreases when they get older. Therefore the interaction between the genetic factors responsible for *SF* sterility leads also to an unusually high level of *X*-chromosome non-disjunction. Another line of evidence has been obtained with the i_0 strain mentioned in the Introduction. Females produced from a cross between i_0 males and females of the *strong* reactive strain seF_8 do not exhibit any significant level of non-disjunction (Pélisson, in preparation).

Another important point which appears from these results is the occurrence of mutations at a higher rate in *SF* females and in some *RSF* females than in control inducer or reactive females. The data do not make it clear whether the mutation

rate depends on the level of reactivity of the cytoplasm inherited by *SF* females and whether it decreases with ageing of these females. Nevertheless the mutation rate is always higher in *SF* females than in *RSF* females of the same genotype. This systematic difference between *SF* and *RSF* females provides evidence that a high rate of mutation is another manifestation of the interaction between the genetic factors responsible for *SF* sterility. The occurrence of some mutations in *SF* females showing only a weak reduction of fertility and in fertile *RSF* females is not in contradiction with this conclusion. It is known (Picard, 1978*c*) that chromosomal contamination, which is another manifestation of the interaction between the *I* and *R* genetic factors, occurs with a noticeable frequency in *SF* females coming from very *weak* reactive strains and in *RSF* females.

Further, it must be noted that the majority of the mutations scored in females (14/15) are associated with recessive lethal mutations. Since in the experiments reported in this paper, *X*-linked mutations could be detected in females at only a few loci, and in males only when they were not recessive lethals, the data highly underestimate the true frequency of *X*-linked mutations. Preliminary results concerning recessive lethals on the *X* chromosomes show that they occur with a high frequency in *SF* female germ lines (Prudhommeau & Proust, personal communication).

The association between these visible mutations and recessive lethality clearly shows that they are not point mutations but rather small deletions. For example, the seven *cut* mutations recovered in females are all associated with a recessive lethal; whereas this is not the case for the spontaneous *cut* mutations reported by several authors (Lindsey & Grell, 1968). Therefore most of the mutability observed in our experiments is probably a result of chromosome breakage.

Another interesting point should be mentioned: some mutations recovered in male progeny, and therefore not associated with recessive lethality appear to be unstable with a reversion frequency ranging from 10^{-3} to 10^{-2} (Pélisson, unpublished data). This connects our results with those of Green (1977) and Golubovsky & Erokhina (1977) and suggests that these mutants may result from insertion events.

It will be important to investigate the relationships between the different types of mutants recovered here and the chromosomal contamination of *I* factor which seems to involve a transposition mechanism (Pélisson, 1978).

It is now necessary to compare these results with those of Kidwell *et al.* (1977*b*) and to take a critical look at previous studies in the field of 'mutator genes' in *Drosophila melanogaster* (see, notably, the review by Green, 1976).

Concerning the relationships between the *I–R* and the *P–M* systems the following points may be noted:

(1) The *P–M* classification has been established mainly on the basis of male recombination and female sterility occurring between 25 and 29 °C. Recent work has indicated that this sterility is the result of gonadal dysgenesis (Schaefer & Kidwell, in preparation). Further clarification of the *P–M* system using this specific character is under way.

(2) Results obtained from crosses between the four categories of strains of the

I–R and *P–M* systems seem to indicate that the two systems are at least causally independent (M. G. Kidwell, in preparation).

(3) It has not yet been demonstrated that all dysgenic traits described by Kidwell *et al.* (1977*b*), notably male recombination and male mutation, are produced in the *I–R* system.

These three points are under active investigation.

The implications of our results for the genetic study of *Drosophila* require a more detailed discussion. Because of the uncertainties pointed out above, this discussion will be mainly in relation to the *I–R* system and will be limited to the field of mutation.

M. G. Kidwell (1975 *et seq.*) has put forward the idea that some previous results, attributed to mutator genes in natural populations of *Drosophila melanogaster*, may be the result of interactions between the chromosomes of lines caught in the wild and the cytoplasm of marked laboratory stocks used in the mating scheme. This hypothesis is supported by the results of Kidwell *et al.* (1977) and by the experiments reported here on the *I–R* interaction. In the light of our knowledge about the complex genetic behaviour of these two factors, it is interesting to make a rapid survey of previous work.

It appears that in most cases chromosomes have been extracted from wild flies in order to test their capacity to induce mutations. Since this extraction has been carried out by the marked inversion technique which requires successive crosses with balanced stocks, it is possible that in many cases a reactive laboratory stock has been used. If this is the case there would be inducer chromosomes and a reactivity level of variable strength in the F_1 females (*SF* or *RSF*). In the following backcrosses with the balanced stock, the reactivity would be maintained or even strengthened by injection of new reactive chromosomes; and inducer chromosomes also would be maintained by means of chromosomal contamination which occurs with high frequency even in *RSF* females (Picard, 1978*a*, *b*). This would be true even if the extracted chromosomes were not themselves inducers before the crosses were made. The flies obtained at the end of this extraction process and tested for mutation frequency would have inducer chromosomes and reactive cytoplasm and therefore a high level of *I–R* induced mutations.

It is also interesting to note the non-reproducibility of the results in several studies. The mutation frequencies often vary over a wide range from one experiment to another and sometimes fall to the control value. The authors generally concluded either that there was variation of the mutator gene or that it was eventually lost. (See, for example, Demerec, 1937; Zuitin & Pavlovetz, 1940; Ives, 1950.) In the hypothesis of inducer-reactive interaction, this non-reproducibility can be readily accounted for by the great range of variation existing for the reactive state. This variation can arise from the following causes:

(1) In a strain which has never been selected for the level of reactivity, great individual variation exists (Picard *et al.* 1972).

(2) Spontaneous genetic drift occurs sometimes from a strong to a weak mean reactivity or the converse (Picard *et al.* 1972; Bucheton, 1973).

(3) The reactivity level of females becomes lower with ageing (Bucheton & Picard, 1975).

(4) This reduction is heritable and can accumulate over several generations. Therefore a particular stock will be more or less reactive if it is generated at each generation from young or old flies (Bucheton, in preparation). This is generally not rigorously controlled in laboratories and can contribute to variations.

From the above considerations it can be emphasized that in all cases where successive crosses with a laboratory strain have been performed, the results obtained may be the consequence of interaction between inducer and reactive strains or between interacting strains of another similar system. The finding of high mutation frequencies in this kind of experiment does not prove at all that a mutator effect exists in wild populations *per se*.

The only good experimental schemes for testing the existence of indigenous mutation systems in natural populations are those which allow the measurement of mutation frequencies in flies whose ancestors had never been crossed with laboratory strains. However, it must be noted that even in this situation, the *I–R* interaction is not completely excluded. Indeed, conventional contamination of a wild inducer stock by reactive flies is possible in the laboratory and this may lead to a transient coexistence of the two factors within the strain.

The question remains whether the above considerations are valid also for other dysgenic traits particularly for male recombination and chromosomal aberrations which are much studied at present by several authors.

Kidwell *et al.* (1977*b*) have demonstrated that male recombination can be induced by strain interactions. Recent unpublished results seem to indicate that it is not associated systematically with the *I–R* interaction and that this dysgenic trait is more likely to be the result of the *P–M* interaction. However, it may be of interest to point out at least one analogy between the MR factor and the *I* factor. When Voelker (1974) and Slatko & Hiraizumi (1975) tried to localize the MR factor they obtained complex results which led them to invoke 'evanescent secondary elements' or 'transposable elements'. These experimental data agree very well with the behaviour of the *I* factor and its chromosomal contamination.

Regarding chromosomal aberrations, we have seen that many mutational events reported in this paper are associated with recessive lethals and at least one of them is associated with a deletion visible on salivary gland chromosomes: it is therefore likely that inversions and translocations are produced by the *I–R* interaction.

Regardless of the uncertainties concerning dysgenic traits other than non-disjunction and mutation in the female germ line, the following suggestions are made with respect to future studies.

All experiments in the field of *Drosophila* genetic abnormalities should be performed with maximum care. The precaution of making reciprocal crosses as suggested by Kidwell *et al.* (1977*b*) in order to detect strain interactions may not be sufficient, at least in the *I–R* system. We know that the interaction exists in *RSF* females, where it produces at least chromosomal contamination by the *I* factor and perhaps a significant level of dysgenic traits if the strains used are

strong inducer and *strong* reactive; furthermore if backcrosses are made to a reactive stock, the interaction becomes stronger (Picard, 1978*a*, *b*).

Thus the only valid mating schemes are those involving strains of the same category. This requires knowledge of the inducer and reactive characters of laboratory stocks used. Here too, careful tests must be performed: it is necessary to cross reciprocally each laboratory stock with both *strong* inducer and *strong* reactive strains, to measure the hatching percentage of eggs laid by hybrid females and its variation with ageing. The cross with *strong* inducer and reactive strains is necessary in order to detect weak inducer efficiency or weak reactive levels. As it appears that the *P–M* system is partially distributionally independent of the *I–R* system (Kidwell, in preparation), similar precautions will be necessary using an easily observable character which will identify the *P–M* interaction.

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