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Hybrid dysgenesis in *Drosophila melanogaster*: partial sterility associated with embryo lethality in the *P–M* system

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SUMMARY

Variable frequencies of unhatched eggs were observed to be produced by a number of F_1 interstrain hybrids. This type of partial sterility resulting from F_2 embryo death was found to be associated with the *P–M* system of hybrid dysgenesis. Dysgenic hybrid progeny of crosses between *M* strain females and *P* strain males may therefore have reduced fertility due to the disruption of development at two different stages: early F_1 gonadal development and early F_2 embryo development. These disruptions result in the previously described F_1 gonadal dysgenesis (*GD* sterility) and F_2 embryo lethality (*EL* sterility) respectively. The two morphologically distinct types of *P–M*-associated sterility differ in their patterns of response to F_1 developmental temperature, and the temperature-sensitive period for *EL* sterility occurs considerably later in F_1 development than for *GD* sterility. *EL* sterility is similar to *SF* sterility, which is associated with the *I–R* system of hybrid dysgenesis in that both result from death during early F_2 embryogenesis. However, *EL* sterility differs from *SF* sterility in not being restricted to hybrids of the female sex and in showing different patterns of response to temperature and ageing in the F_1 generation. Some implications of the existence of *EL* sterility for methods of strain classification in the *I–R* system are explored.

1. INTRODUCTION

The phenomenon of hybrid dysgenesis in *D. melanogaster* (Kidwell, Kidwell & Sved, 1977) is associated with the mobilization of two families of transposable elements, the *P* elements and the *I* elements (Bregliano & Kidwell, 1983). Strains characterized as being of the *P* or *Q* types, on the basis of their functional properties, carry 30–50 copies of the *P* element family. In the germ line, these elements are susceptible to destabilization in the maternal cytoplasm (cytotype) of *M* strains which do not carry *P* elements (Engels, 1979; Bingham, Kidwell & Rubin, 1982; Rubin, Kidwell & Bingham, 1982). *I* factors are carried by *I* strains and are susceptible to destabilization in the reactive cytoplasm of *R* strains. There is considerable evidence that the *P–M* and *I–R* systems of hybrid dysgenesis are independent of one another (Kidwell, 1979; Bregliano & Kidwell, 1983).

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F_1 hybrids produced from matings between *M* strain females and *P* strain males and from matings between *R* strain females and *I* strain males are prone to the induction of a number of associated aberrant genetic traits. In the *P–M* system, dysgenic traits occur in hybrids of both sexes and include male recombination, gonadal (*GD*) sterility, mutation and chromosomal aberrations. In the *I–R* system, hybrid dysgenesis is limited to females and is manifested as *SF* sterility, mutation, non-disjunction and chromosomal aberrations (Bregliano *et al.* 1980; Bregliano & Kidwell, 1983).

While some dysgenic traits such as mutation and chromosomal aberrations appear to be common to both systems of hybrid dysgenesis, others, such as gonadal sterility and male recombination, are found to be associated with the *P–M* system, but not the *I–R* system. Likewise, it had earlier been considered that the reduced hatchability of F_2 eggs typical of *SF* sterility was limited to the *I–R* system (Picard *et al.* 1977). However, with the exception of a preliminary short report (Kidwell, 1983*a*), no systematic study of egg hatchability in $P \times M$ hybrids in the absence of the *I–R* interaction has been reported previously. This paper describes such an investigation and provides evidence of F_2 reduced hatchability which is unambiguously associated with the *P–M* interaction and which has both similarities and differences with *SF* sterility. The possible implications of these results for several aspects of hybrid dysgenesis are discussed.

2. MATERIALS AND METHODS

(i) *Strains*

Canton-S (*IM*)	Luminy (*IP*)
Cockaponsett Forest (*RM*)	Mount Carmel (*IQ*)
Cranston (*IP*)	Nettlebed (*IM*)
Florida (*IM*)	Oxford (*IP*)
Harwich (*IP*)	*se* F_8 (*RM*)
Inhaca (*IP*)	Tottori (*IQ*)
Kaduna (*IM*)	731 C (*IM*)

The letters in parentheses indicate the designation of each strain in the *I–R* and *P–M* systems of hybrid dysgenesis respectively, based on previously established criteria from *SF* and *GD* sterility tests (Picard *et al.* 1977; Kidwell, 1979). Further details of these strains are provided by Kidwell (1979) and Kidwell, Frydryk & Novy (1983).

(ii) *Production of dysgenic hybrids*

Mass matings were made between 20–30 males and virgin females of each of the selected parental strains. In accordance with previously established usage (Kidwell, 1979), the dysgenic crosses between $M♀♀$ and $P♂♂$, or between $R♀♀$ and $I♂♂$, are referred to as Cross A in the *P–M* and *I–R* systems, respectively. The reciprocal control crosses between $P♀♀$ and $M♂♂$, or between $I♀♀$ and $R♂♂$, are referred to as Cross B.

(iii) *Measurement of hatchability*

Appropriate mass matings were made between parental strains and the F_1 generation was developed at various temperatures chosen according to the purpose of the specific experiment. Unless otherwise stated, F_1 males and females were allowed to mate *inter se*. Ten F_1 individuals of each sex aged 2–3 days were placed in half-pint milk bottles. The bottles were inverted on to cardboard caps carrying an agar–grape juice medium, seeded with live yeast, for egg collection. The caps were replaced every 24 h. Eggs were counted immediately after the caps were removed from the bottles. The caps were held for a further 48 h in Petri dishes lined with moist filter paper. The number of unhatched eggs was then counted.

(iv) *Method of measurement of ovarian dysgenesis*

Mass matings were made between the parental strains under test, and the F_1 generation was allowed to develop at 20, 25 or 29 °C. F_1 hybrid females were aged for 2–3 days and their ovaries were dissected in water. The frequency of ovarian dysgenesis was estimated according to the methods of Schaefer *et al.* (1979) and Kidwell *et al.* (1983).

(v) *Measurement of egg production*

Mass matings were made between the parental strains under test and the F_1 generation developed at 20, 25 or 29 °C. F_1 females were individually mated with two males from the Canton-S strain in batteries of small plastic tubes. The tubes were inverted on to Petri dishes to which $\frac{1}{4}$ in of a grape juice–agar medium seeded with live yeast had been added. The Petri dishes were replaced every 24 h and the egg production of individual females was recorded.

3. RESULTS

(i) *Interaction between hybrid dysgenesis and temperature*

The design of the first set of experiments was motivated by the results of Eggleston & Kearsey (1980), who claimed that there was a high degree of correlation in the response of *SF* and *GD* sterilities to various developmental temperature regimes. Accordingly, these experiments were performed to clarify the effect of developmental temperature on three specific traits induced in the F_1 progeny of known *P–M* and *I–R* dysgenic crosses. The traits were (*a*) hatchability of eggs laid by F_1 dysgenic hybrids, (*b*) ovarian dysgenesis in F_1 dysgenic females (*GD* sterility) and (*c*) egg production of F_1 dysgenic females.

Three types of dysgenic cross were made:

Cross E: *RM*♀♀ × *IP*♂♂.

This type of cross is dysgenic for both the *I–R* and *P–M* systems and was represented by two independent matings:

E 1: *se* F_8 ♀♀ × Harwich ♂♂,

E 2: Cockaponsett Forest ♀♀ × Cranston ♂♂.

Cross F: *IM*♀♀ × *IP*♂♂.

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This type of cross is dysgenic for the *P–M* system but not for the *I–R* system and was represented by two matings:

F 1 : Canton-S ♀♀ × Harwich ♂♂,

F 2 : Canton-S ♀♀ × Cranston ♂♂.

Cross G: *RM* ♀♀ × *IM* ♂♂.

This type of cross is dysgenic for the *I–R* system but not for the *P–M* system. It was represented by two matings:

G 1 : *se* F_8 ♀♀ × Canton-S ♂♂,

G 2 : Cockaponsett Forest ♀♀ × Canton-S ♂♂.

The methods of measurement of the three traits are described in Materials and Methods. One of three developmental temperatures, 20, 25 or 29 °C, was employed throughout the entire developmental period of F_1 hybrids (i.e. from the time of parental mating to F_1 eclosion).

(*a*) *F_2 hatchability*

The effect of developmental temperature on the frequency of F_2 unhatched eggs is illustrated in Fig. 1. *A priori* it was expected that only the crosses of types E and G would show elevated frequencies of unhatched eggs due to *SF* sterility associated with the *I–R* system (Picard *et al.* 1977), but that the type F crosses would have normal hatchability. However, all three types of crosses showed reduced hatchability, with the E crosses tending to be the most extreme in this respect. The unexpected observation of reduced hatchability in both the F crosses suggested the possibility that this trait might not be restricted to *I–R* dysgenesis but might also be associated with *P–M* dysgenesis.

(*b*) *Ovarian dysgenesis*

The frequencies of ovarian dysgenesis in F_1 females of the three types of crosses are given in Fig. 2. As expected, *I–R* dysgenesis alone (G crosses) showed no ovarian dysgenesis. The E and F crosses showed significant frequencies of dysgenic ovaries at high developmental temperatures but not at 20°. This result was also as expected due to the temperature sensitivity of *GD* sterility associated with the *P–M* system (Engels & Preston, 1979; Kidwell & Novy, 1979).

(*c*) *Egg production*

The egg production of 30 individual F_1 females per cross was measured for three days and the ovaries of these females were then dissected. The mean three-day egg production of the six crosses is illustrated in Fig. 3. Females that were sterile due to the absence of developed ovaries were eliminated from the egg-production computations. Egg production from all crosses was low at 20 °C. At higher temperatures the production of the G crosses was considerably greater than that of the E and F crosses. These results confirm earlier findings that egg production is reduced in *P–M* dysgenic hybrids (Broadhead *et al.* 1977; Engels & Preston, 1979) and that this reduction cannot be directly related to the presence of rudimentary gonads.

In summary, the results of this experiment to determine the effects of temperature on three dysgenic traits were in accordance with previous findings with one

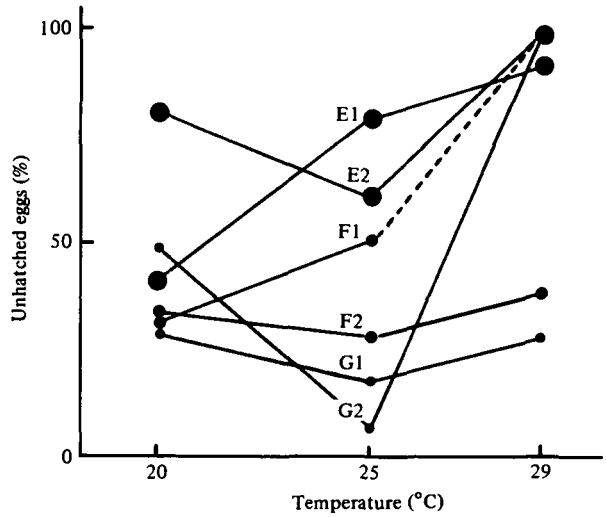

Fig. 1. The effect of F_1 developmental temperature on the frequency of F_2 unhatched eggs in three types of dysgenic crosses: E = *RM*♀♀ × *IP*♂♂; *F* = *IM*♀♀ × *IP*♂♂; *G* = *RM*♀♀ × IM♂♂. See text for details of specific strains used to make the crosses.

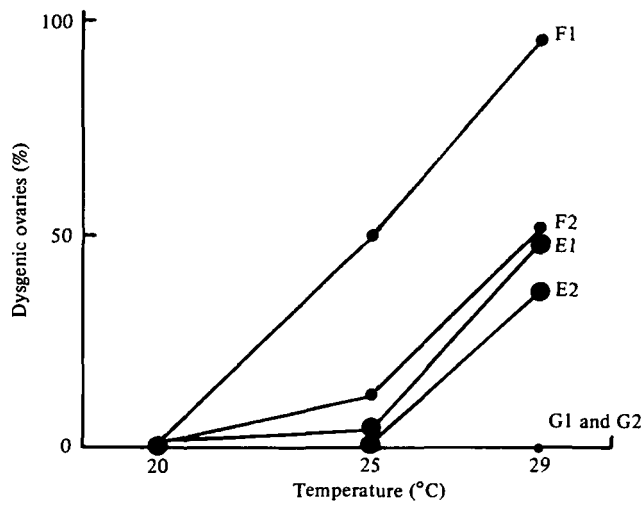

Fig. 2. The effect of F_1 developmental temperature on the frequency of ovarian dysgenesis (*GD* sterility) in three types of dysgenic crosses: *E* = *RM*♀♀ × *IP*♂♂; *F* = *IM*♀♀ × *IP*♂♂; *G* = *RM*♀♀ × *IM*♂♂.

exception: the observation of embryo lethality associated with the type F crosses. The following experiments were designed to examine this embryo lethality (hereafter referred to as *EL* sterility) in greater detail.

(ii) *Test of two hypotheses to account for reduced hatchability of hybrid progeny*

Two hypotheses were formulated to account for the reduced hatchability observed in the eggs laid by hybrids produced from the F cross matings in section (i) above: (*a*) that this is an additional manifestation of the *P–M* system of hybrid

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dysgenesis, (*b*) that it is associated with the addition of live yeast to the culture medium to stimulate egg production (Robertson & Sang, 1944; Minato, 1979).

In order to test the first hypothesis, the following *IM*♀♀ × *IP*♂♂ dysgenic crosses and their reciprocals were established: Kaduna♀♀ × Harwich♂♂, 731C♀♀ × Harwich♂♂, 731C♀♀ × Cranston♂♂, Florida♀♀ × Cranston♂♂, Florida♀♀ × Oxford♂♂, Kaduna♀♀ × Inhaca♂♂, Nettlebed♀♀ × Inhaca♂♂, Nettlebed♀♀ × Oxford♂♂. Thirty F_1 females, aged three days after eclosion, were each individually mated to two of their brothers and the hatchability of all eggs laid over three 24 h periods was scored. The temperature was held at 25 °C throughout the experiment.

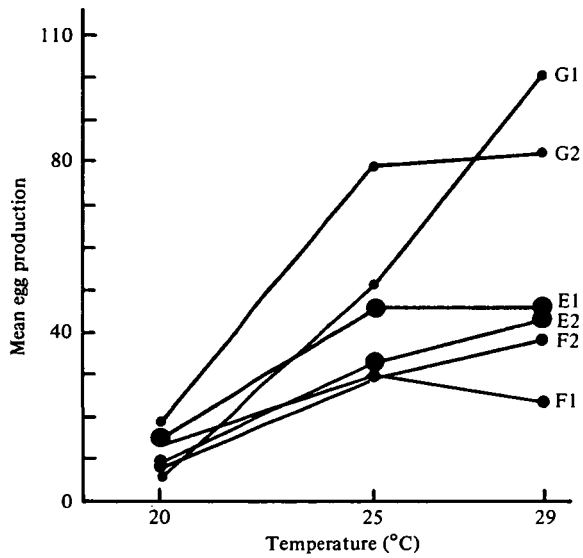

Fig. 3. The effect of F_1 developmental temperature on mean egg production in three types of dysgenic crosses: *E* = *RM*♀♀ × *IP*♂♂; *F* = *IM*♀♀ × *IP*♂♂; *G* = *RM*♀♀ × *IM*♂♂.

In order to test the second hypothesis, the above sets of matings were made and tested in duplicate. Live yeast was not added to the culture medium of the first group at any stage of the experiment. In contrast, live yeast was liberally added to the cultures of the second group.

The results are presented in Table 1. There were marked increases in the frequencies of unhatched eggs laid by the *P–M* dysgenic hybrids (Cross A) compared to those laid by the reciprocal control hybrids (Cross B). The difference between Cross A and B distributions is illustrated in Fig. 4 for three of the no-yeast crosses. These results suggest a strong association between reduced hatchability and *P–M* hybrid dysgenesis. There was, however, large variation in the mean hatchability reduction of different Cross A hybrids in comparison with the reciprocal B crosses. Overall, there was no consistent effect of the yeast treatment on egg hatchability, although for one or two individual crosses a tendency for yeast to reduce hatchability was suggested.

Table 1. *Mean frequencies of unhatched eggs produced by $F_1 \times F_1$ single-pair matings from reciprocal crosses A ($IM♀ \times IP♂$) and B ($IP♀ \times IM♂$)*

	No yeast		Yeast	
Cross	A	B	A	B
Kaduna × Harwich	67·7 ± 3·81	5·8 ± 1·48	58·9 ± 6·91	3·4 ± 1·15
731 C × Harwich	54·0 ± 5·27	6·2 ± 1·48	70·5 ± 5·51	3·8 ± 1·18
731 C × Cranston	25·6 ± 1·90	2·6 ± 1·05	35·3 ± 2·73	1·7 ± 0·78
Florida × Cranston	10·7 ± 1·66	1·5 ± 0·55	13·9 ± 2·38	2·3 ± 0·62
Florida × Oxford	7·9 ± 1·50	3·4 ± 1·55	22·0 ± 3·96	4·2 ± 1·28
Kaduna × Inhaca	45·0 ± 4·11	1·9 ± 2·98	42·0 ± 3·33	1·3 ± 0·63
Nettlebed × Inhaca	21·7 ± 2·71	0·9 ± 0·36	24·9 ± 2·51	1·9 ± 0·42
Nettlebed × Oxford	36·1 ± 2·32	0·8 ± 0·25	48·4 ± 3·86	6·4 ± 2·04
Mean	33·6 ± 7·45	2·9 ± 0·74	39·5 ± 6·83	3·1 ± 0·60

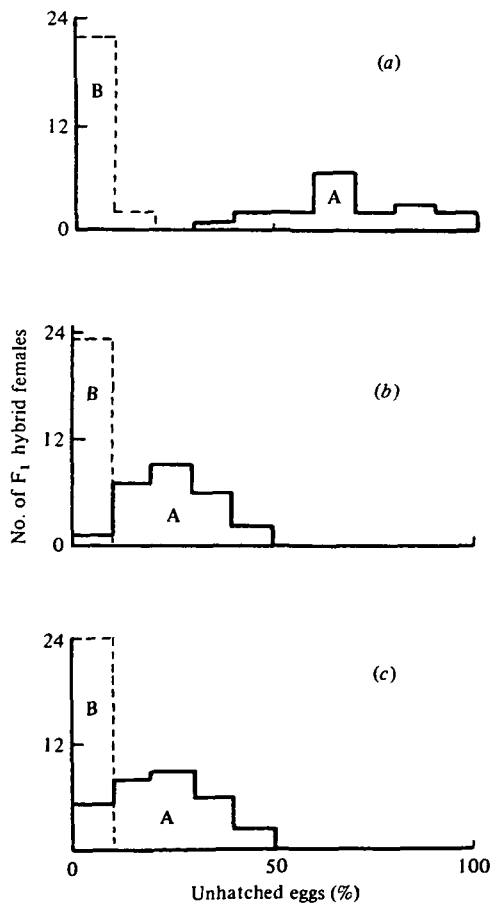

Fig. 4. The distributions of unhatched egg frequencies laid by dysgenic hybrid females (A) and non-dysgenic hybrid females (B). The crosses were (*a*) Kaduna × Harwich, (*b*) 731 C × Cranston and (*c*) Florida × Cranston.

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(iii) *Strain cross-correlation of F_2 reduced hatchability with F_1 ovarian dysgenesis*

In the above experiment, carried out at 25 °C, a few females did not produce eggs because of ovarian dysgenesis. In order to observe the relationship between F_2 reduced hatchability and maximum ovarian dysgenesis, some of the crosses made in section (ii) above were repeated, but the F_1 developmental temperature was 29 instead of 25 °C. Two additional $Q \times M$ matings were also included in the 29 °C test. A sample of 50 F1 females was dissected from each cross following ageing for at least two days after eclosion. A comparison of the frequencies of ovarian dysgenesis and unhatched eggs for these crosses is presented in Table 2, and the relationship is graphed in Fig. 5. With one exception, the results suggest a linear association between ovarian dysgenesis (measured at 29 °C) and the frequency of reduced hatchability (measured at 25 °C).

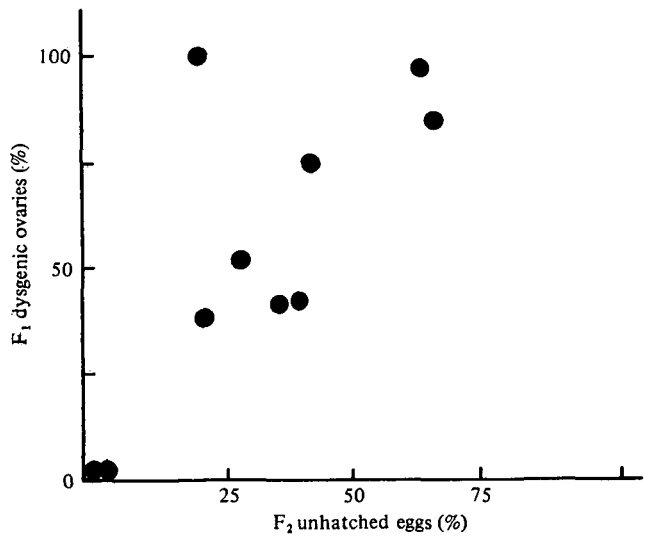

Fig. 5. The relationship between F_1 dysgenic ovary frequency (*GD* sterility) and the frequency of F_2 unhatched eggs (*EL* sterility) in ten *P–M* dysgenic crosses.

(iv) *Effect of sex on EL sterility*

In the previous experiments, F_2 hatchability was measured in the eggs of F_1♀ × F_1♂ matings. It was not possible from this design to determine whether one or both sexes of F_1 hybrids contributed to F_2 lethality. Accordingly, several reciprocal crosses were performed, and for each the F_1 dysgenic male and virgin female hybrids were mated separately with non-dysgenic partners from a different stock (Canton-S). The hatchability of eggs was scored in the normal way. The results are presented in Table 3. There were significant levels of embryo lethality in the progeny of dysgenic parents of both sexes relative to the reciprocal control crosses. This result contrasts sharply with the occurrence of *SF* sterility, associated with the *I–R* system which is completely determined by the female hybrid. The male contribution to *EL* sterility often exceeded that of the female but it is not clear that there is a consistent, significant sex difference. However, a sex difference

Table 2. *Relationship between frequency of F_1 ovarian dysgenesis* (GD *sterility*) *and freqency of unhatched eggs in F_1 hybrids from various reciprocal crosses*

	% F_1 rudimentary ovaries (29 °C)		% F_2 unhatched eggs (25 °C)	
Parental cross	A	B	A	B
Kaduna × Harwich	86·0	0	64·3	6·4
731 C × Harwich	97·9	0	62·5	5·8
731 C × Cranston	40·0	0	30·1	3·5
Florida × Cranston	35·9	0	11·9	2·0
Florida × Oxford	100·0	0	22·0	3·9
Kaduna × Inhaca	42·0	0	42·6	1·8
Nettlebed × Inhaca	52·8	0	21·0	1·3
Nettlebed × Oxford	75·4	0	40·1	3·4
Kaduna × Tottori	1·2	0	2·6	0·1
Canton × Mt Carmel	1·5	0	3·6	1·3

Table 3. *Effect of sex of F_1 hybrids on frequencies of unhatched eggs produced by the progeny of dysgenic $M♀ \times P♂$ crosses (A) and their $P♀ \times M♂$ reciprocals (B) at two developmental temperatures*

		% unhatched eggs			
		Cross A		Cross B	
Parental strains	Developmental temperature	$F_1♀$	$F_1♂$	$F_1♀$	$F_1♂$
Canton-S × Harwich	23 °C	10·9	35·2	0·9	5·8
Kaduna × Harwich		24·7	23·2	4·8	4·0
Kaduna × Inhaca		9·8	21·5	2·1	2·4
Canton-S × Harwich	25 °C	52·5	44·4	0	8·8
Kaduna × Harwich		53·5	68·8	3·4	17·9
731 C × Harwich		53·1	96·8	1·6	10·2
731 C × Cranston		4·7	48·2	6·4	8·7
Nettlebed × Cranston		6·3	31·6	2·3	24·9
Nettlebed × Oxford		22·0	39·4	0·9	21·7
Kaduna × Oxford		21·8	45·8	0·9	29·1
Kaduna × Luminy		4·6	17·6	5·4	7·5
Canton-S × Luminy		6·4	13·2	3·1	11·1

in embryo lethality from the B cross matings made at 25 °C was noted; there tends to be a relatively low but significant frequency of unhatched eggs in the progeny of *B* cross males, but females from the same B cross lay eggs whose hatchability is unimpaired.

In a related experiment, it was determined whether the *P* or *M* nature of the strain used for crossing with F_1 hybrids might affect egg hatchability. Reciprocal crosses between the Canton-S and Harwich strains were made as before. F_1 male and virgin female hybrids from both the dysgenic (A) and non-dysgenic (B) parental crosses were backcrossed with both parental strains and the frequency of embryo lethality in their progeny was determined. The results of F_2 hatchability tests are given in Table 4. In both dysgenic and non-dysgenic crosses, the Harwich backcross gave a hatchability a few percentage points lower than the Canton-S

backcross. However, hatchability of the dysgenic crosses was in all cases considerably lower than the appropriate reciprocal crosses, and the reduction of hatchability of eggs with a paternal dysgenic contribution was about twice that of eggs with a maternal dysgenic contribution.

Table 4. *Effect of strain to which F_1 hybrid was backcrossed on frequency* (%) *of unhatched eggs. The parental matings were Canton-S♀ × Harwich♂ (Cross A) and Harwich♀ × Canton-S♂ (Cross B)*

Strain of F_1 hybrid mate	Sex of F_1 hybrid			
	♀		♂	
	A	B	A	B
Canton-S	11·8	0·8	28·3	8·5
Harwich	15·7	2·9	34·3	11·7

Over 500 eggs were examined for each cross. The F_1 hybrids developed at 23 °C.

(v) *Effect of temperature and ageing of F_1 hybrids*

Temperature and F_1 female age have important influences on the frequency of *SF* sterility (Bucheton, 1979). It was therefore of interest to know whether these factors affect *EL* sterility. The effects of developmental temperature (i.e. the temperature from egg laying to adult eclosion), test temperature (i.e. the temperature at which F_1 hybrids were mated and at which egg laying occurred) and F_1 hybrid age were investigated in a series of experiments. Mass matings of the eight *P–M* dysgenic crosses previously tested in section (ii) above (Cross type A) were set up in bottles and allowed to develop at either 20 or 25 °C until eclosion of the F_1 adults. Male and female F_1 hybrids were mated *inter se* and placed on grape juice caps in bottles at about 2–3 days of age. The temperature at which eggs were laid and F_2 egg hatchability observed was either 20 or 25 °C. The adult flies were transferred to fresh grape juice caps every 24 h for a total of 26 days. Hatchability of eggs was scored between 48 and 72 h after egg laying.

Fig. 6 illustrates the comparative effects on hatchability of 20 and 25 °C F_1 developmental temperatures over an egg-laying period of 26 days. The plotted values are the mean percentage of unhatched eggs for all eight crosses tested. The F_1 hybrids in both groups were held at 20 °C throughout their egg-laying period. The frequency of unhatched eggs laid by dysgenic hybrids which had developed at 20 °C was low, particularly in older hybrids. In contrast, development at 25 °C increased the frequency of unhatched eggs, which for most ages was between 25 and 50 per cent. There was considerable day-to-day variability in hatchability, especially in later stages of the experiment, possibly reflecting the small numbers of eggs laid by older females. Thus, in contrast to the clear effect of F_1 developmental temperature on F_2 egg hatchability, there was no clear effect of F_1 age on this trait.

Fig. 7 shows egg hatchability measured for two groups of hybrids in the same way as for Fig. 6 except that one group was held at 25 °C over the 26-day period in which egg hatchability was measured and the other group was held at 20 °C.

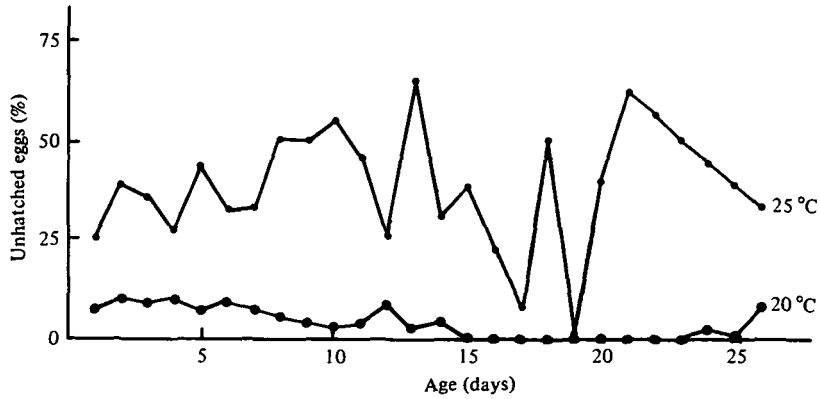

Fig. 6. The effect of F_1 age and developmental temperature on the frequency of *EL* sterility. The data are mean percentages of unhatched eggs laid by eight *P–M* dysgenic crosses (see text for details). The temperature for egg laying was 20 °C for both groups of hybrids.

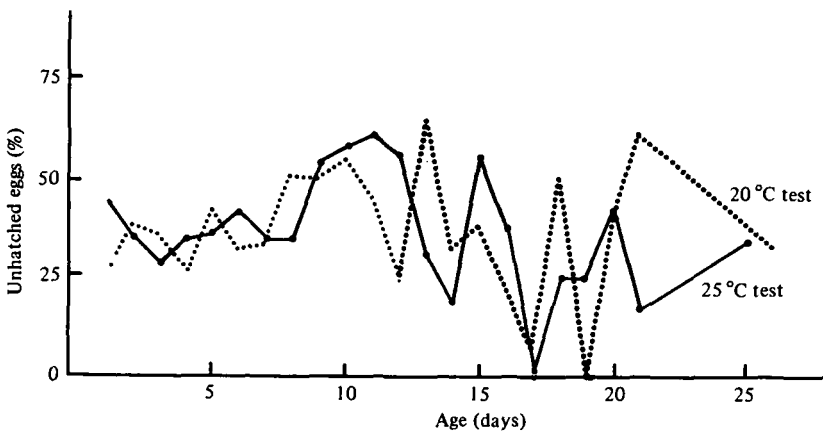

Fig. 7. The effects of age and F_2 egg-laying temperature on the frequency of *EL* sterility. The F_1 developmental temperature was 25 °C for both groups of hybrids.

The F_1 developmental temperature was 25° for both groups. The similarity of the hatchability values for the two groups throughout the period strongly suggests that test temperature has no effect on F_2 egg hatchability.

In Fig. 8 the response of F_2 egg hatchability to increasing F_1 developmental temperature is plotted. Reciprocal parental crosses were made between the *IM* strain Kaduna and the *IP* strain, Inhaca. Representatives of each of the dysgenic (A) and non-dysgenic reciprocal (B) crosses were raised from egg to adult at six developmental temperatures. Male and female F_1 hybrids were then allowed to mate *inter se*, and hatchability was measured at a test temperature of 25 °C. The hatchability of eggs laid by dysgenic hybrids decreased steadily with increasing developmental temperature. In contrast, the reciprocal, control cross hatchability remained at a high level at all developmental temperatures. These results clearly establish the temperature dependence of *EL* sterility. In contrast to *GD* sterility (Kidwell & Novy, 1979), the relationship between *EL* sterility and temperature appears to be approximately linear over the measured range.

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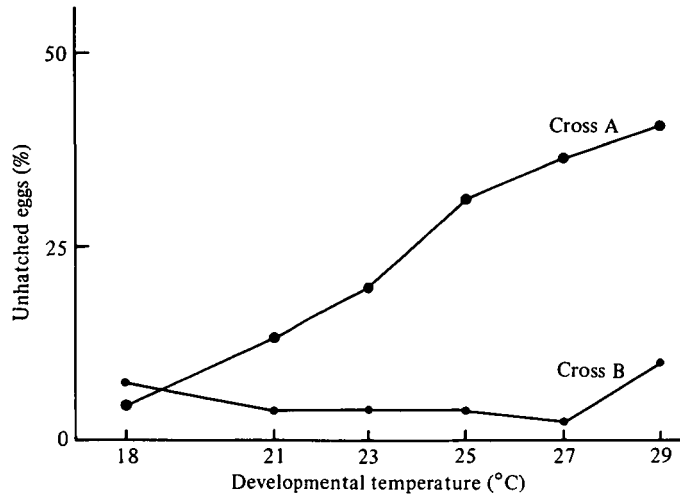

Fig. 8. Temperature response curve for *EL* sterility. The dysgenic (A) cross was Kaduna♀♀ × Inhaca♂♂. The control (B) cross was Inhaca♀♀ × Kaduna♂♂.

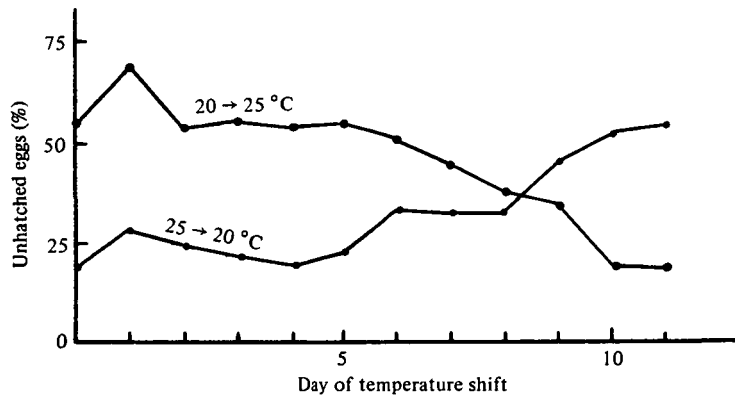

Fig. 9. Percentage of unhatched eggs in the F_1 progeny of crosses between Canton-S females and Harwich males subjected to a shift from 25 to 20 °C temperatures and vice versa. The indicated day of temperature shift was adjusted to the equivalent day at 25 °C in order to equate stages of development.

(vi) *Determination of temperature-sensitive period*

In order to determine the F_1 stages of hybrid development that are sensitive to temperature, developing progeny of a potentially dysgenic cross were switched at different stages from 'restrictive' (25 °C) to 'permissive' (20 °C) temperatures, and vice versa. Parental crosses between 25 Canton-S virgin females and 25 Harwich males were used for each temperature treatment. Half the number of cultures were started at 25 °C, and after successively longer periods of development, individual cultures were 'switched down' to 20 °C to complete their development. (On the assumption that temperature has a linear effect on development time, each time increment was calculated to be equivalent to 24 h at 25 °C.) The remaining cultures were started at 20 °C and 'switched up' to 25 °C in a similar way.

Two separate experiments were conducted, employing the same crosses and a similar design. In the first experiment (Fig. 9), 30 F_1 females were mated with 30 F_1 males in each treatment class. The critical temperature-sensitive phase appeared to exist during the latter half of the developmental period, between the fifth and tenth day. This result is in marked contrast to the temperature-sensitive stage for gonadal sterility, which occurred before the fourth day of development (Engels & Preston, 1979; Kidwell & Novy, 1979).

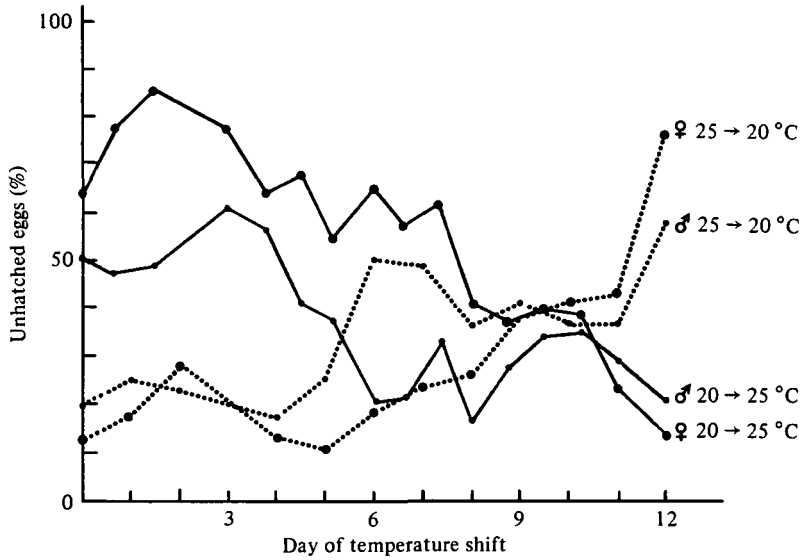

Fig. 10. Results of temperature shift measured separately for F_1 male and female hybrids. Crosses and conditions are otherwise the same as for Fig. 9.

In the second experiment (Fig. 10) the temperature-sensitive stage for F_1 males and females was tested separately. F_1 male and virgin female hybrids were mated with virgin females and males respectively from the Canton-S stock. Otherwise the design was identical to that of the previous experiment. The results provide some indication that the temperature-sensitive stage may start 2–3 days earlier in male hybrids than in female hybrids. This observation suggests that temperature sensitivity may coincide with the stage of meiosis, which occurs several days earlier in males than females (Bodenstein, 1950).

4. DISCUSSION

The results reported here demonstrate that reduced hatchability of various degrees may occur in the progeny of *P–M* dysgenic F_1 hybrids. Such hybrids, therefore, appear to be susceptible to reduced fertility due to disruption of development at different stages. First, if they are subjected to high temperatures early in development there is a chance that both F_1 male and female hybrids will be sterile due to rudimentary gonads (*GD* sterility) (Kidwell & Novy, 1979; Engels & Preston, 1979). If the germ-line cells of these *P–M* hybrids survive the hazard

of *GD* sterility, as a result of a low developmental temperature or an inherent low level of *P* factor activity of the paternal *P* strain, it seems they may be susceptible to embryo lethality in the F_2 generation (*EL* sterility).

EL sterility associated with the *P–M* system of hybrid dysgenesis is superficially very similar to *SF* sterility associated with the *I–R* system. Both types of sterility result from the death of embryos in the progeny of dysgenic hybrids, and their manifestation, like other dysgenic traits, is essentially non-reciprocal. Furthermore, like *SF* sterility (Picard *et al.* 1977) the arrest of F_2 development in *EL* sterility seems to occur very early at the time of late cleavage (A. Fausto-Sterling, personal communication). However, as demonstrated above, there are some important differences between the *SF* and *EL* types of sterility. *SF* sterility is restricted to F_1 dysgenic females whereas *EL* sterility is manifested in hybrids of both sexes. Furthermore, *SF* sterility decreases with the age of F_1 females, whereas *EL* sterility shows no consistent change with increasing parental age. *SF* sterility decreases if the female egg-laying temperature is increased (Picard *et al.* 1977), whereas the test temperature does not affect the frequency of *EL* sterility. However, the effect of developmental temperature is similar for both types of sterility; as with *SF* sterility (Bucheton, 1979) the frequency of *EL* sterility increases with increased developmental temperature.

Discovery of the existence of *EL* sterility raises again the question of the nature of the mechanism(s) responsible for sterility production in hybrid dysgenesis. Because of the extreme nature of developmental disruption it is more difficult to understand the role of the transposition process in producing sterility than in other dysgenic traits such as mutations and chromosomal aberrations, which are usually relatively low-frequency observations. The involvement of specific loci in any type of dysgenesis-induced sterility appears to be unlikely because of the high frequencies often observed and the constancy of the sterility phenotypes. Engels (1983) suggests that *GD* sterility results from 'a specific temperature-sensitive interaction between the metabolism of rapidly dividing germ cells and some aspect of the *P* factor's transposition process'. This idea might be extended to the proposal that the germ line is unequally sensitive to disruption by *P* (or *I*) element-encoded enzymes at different developmental stages. Sterility of different types may result from disruption at different sensitive stages. Further, the stage of late cleavage in embryo development may be a particularly sensitive stage responding to the destabilization of both *P* and *I* elements. The response may be dominant lethal production due to massive chromosome fragmentation similar to that reported by Lavige & Lecher (1982) for *SF* sterility.

Picard *et al.* (1977) assumed that the non-reciprocal embryo mortality referred to as *SF* sterility was specific for and diagnostic of the *I–R* system of hybrid dysgenesis. The results reported here do not directly contradict this assumption because clear differences between *SF* and *EL* sterility can be demonstrated using the appropriate experimental protocols. However, because the standard procedure for characterizing strains in the *I–R* system (e.g. Bucheton *et al.* 1976) does not generally provide the opportunity to differentiate between *SF* and *EL* sterility on the basis of sex, test temperature and ageing effects, the existence of *EL* sterility may have implications for the correct characterization of strains within the *I–R*

system. Because of the superficial similarities between the manifestations of *SF* and *EL* sterilities, it is possible that misclassification of strains with respect to the *I–R* system may have occurred with the use of the standard *SF* protocol.

Table 5 presents a summary of the expected classification of strains, with different combinations of dysgenic potential, on the assumption that reduced hatchability is a specific indicator of *SF* sterility (Picard *et al.* 1977). In Cross A of the *SF* test, males of an unknown strain are mated with females of a reference *RM* strain. In Cross A*, males of the strong inducer reference strain, Luminy, are mated with females of an unknown strain. Luminy has been characterized as weak *P* or *Q* in the *P–M* system. An *I* strain is expected to produce reduced F_2 hatchability in Cross A but not in Cross A*. An *R* strain is expected to give reduced F_2 hatchability in Cross A* but not in Cross A. A neutral (*N*) strain gives normal hatchability in both crosses.

Table 5. *Summary of the likely characterizations of strains with different combinations of* I–R *and* P–M *dysgenic potential on the basis of the standard test for* SF *sterility*

True nature of strain	Type of F_2 sterility expected		Likely previous *I–R* characterization	Existence of discrepancy
	Cross A	Cross A*		
RM	None	*SF*†	*R*	No†
RQ	None	*SF*	*R*	No
RP	EL‡	*SF*	Ambiguous‡	?
NM	None	None†	*N*	No†
NQ	None	None	*N*	No
NP	*EL*‡	None	*I*‡	Yes
IM	*SF*	None†	*I*	No†
IQ	*SF*	None	*I*	No
IP	*SF* + *EL*‡	None	*I*‡	No

† Expectations based on the assumption that Luminy (the standard *I* reference stock) has too low a level of *P* factor activity to produce significant frequencies of *EL* sterility.

‡ Expectations based on the assumption that the tested stock has the *P* factor potential to produce significant frequencies of *EL* sterility.

The Table 3 data provide good grounds for assuming that the potential for *EL* sterility of the Luminy reference strain is too low to provide significant sterility frequencies. There is therefore essentially only one strain combination in which previous results might be questioned in the light of the existence of *EL* sterility. *RP* strains have not previously been reported to exist in either laboratory or wild populations, although strains which behave functionally as *RP* have recently been synthesized in the laboratory (M. G. Kidwell, unpublished results). *RP* strains are expected to give ambiguous results in the standard *SF* test. As indicated in Table 5, sterility is expected to be observed in both A and A* crosses given that the *P* factor activity of the *RP* strain is high enough to produce *EL* sterility. However, ambiguous results of this type have not been reported in combination with an independent test result indicating *P* factor activity.

The main category of real concern with respect to possible *I–R* system misclassification is the putative *NP* class, which has not been observed in an

extensive strain survey (Kidwell, 1983*b*; Kidwell *et al.* 1983). If the *P* factor activity of such a putative strain were strong enough, a significant frequency of sterility in the Cross A *SF* test (due to *EL* sterility) might result in the misclassification of a true *NP* strain as an *IP* strain.

We have no way of knowing at this time whether such *NP* strains exist in laboratory cultures or natural populations. It is likely that in the future the use of *I*- and *P*-element molecular probes will allow an unambiguous characterization of strains. The presence of *P* factors can now be determined using molecular probes (Bingham *et al.* 1982). Also, an internal *I* factor fragment has been identified which hybridizes considerably more strongly with DNA from *I* strains than that from *R* strains (H. M. Sang and D. J. Finnegan, personal communication). However, the use of functional genetic tests for *SF* sterility may continue to be necessary under certain circumstances. These tests might be improved in two ways to avoid ambiguities and incorrect interpretations: (1) by the use of an *RP* rather than a *RM* strain as the reference female in the parental A cross; (2) by the use of an *IM* rather than an *IP* strain as the reference male in the parental A* cross.

Although, theoretically, misclassification is possible as described above, it seems unlikely that the extent of it could be of a significant magnitude for serious concern. Because the rate of transposition of *I* elements is extremely high (Bregliano *et al.* 1980), they would be expected to be highly invasive in natural populations. It is questionable whether many populations exist that could escape such an invasion. There is also the possibility, however, that *I* strains might lose their *I* elements by stochastic loss or other mechanisms (Engels, 1981). The existence of *NP* strains in laboratories and in isolated natural populations might therefore sometimes be expected.

The principal motivation for carrying out the initial experiments described in this paper was to attempt to resolve an apparent discrepancy between the results of Eggleston & Kearsey (1980) and those of previous reports concerning dysgenic traits associated with the *I–R* and *P–M* systems (e.g. Kidwell, 1979). On the basis of high degrees of correlation in the response of two dysgenic traits to various developmental temperature regimes, Eggleston & Kearsey (1980) claimed that *SF* and *GD* sterilities had a common causation and resulted from the same nuclear–cytoplasmic interaction. They suggested that the independence of the two systems of hybrid dysgenesis was therefore thrown into question.

Eggleston & Kearsey's results can readily be explained in the light of the present findings if we accept the likely assumption that their dysgenic crosses were of the *RM* (or *NM*)♀♀ × *IP*♂♂ combination. Reduced F_2 hatchability would be expected due to either *SF* and *EL* sterilities occurring together or to *EL* sterility alone, depending on whether the female parents were reactive or neutral in the *I–R* system. Reduced egg production does appear to be a dysgenic trait associated with the *P–M* system (although we do not accept that it is completely equivalent to *GD* sterility as implied by Eggleston & Kearsey). Thus a correlation of two dysgenic traits, *EL* sterility and reduced egg production, associated with the same system, is not surprising. In retrospect, because they apparently incorrectly equated reduced hatchability with *SF* sterility, Eggleston & Kearsey's results provided no information on the independence of the two systems. More recently,

strong evidence has been provided, using molecular techniques, that *P* and *I* elements have no common homology (Bingham *et al.* 1982; Finnegan, Bucheton & Sang, 1983). Other lines of evidence supporting the independence of the *P–M* and *I–R* systems are reviewed by Bregliano & Kidwell (1983).

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