Genet. Res., Camb. (1984), **44**, *pp.* 251–259
With 5 text-figures
Printed in Great Britain

Hybrid dysgenesis in *Drosophila melanogaster*: the evolution of mixed *P* and *M* populations maintained at high temperature

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(*Received 24 April 1984*)

SUMMARY

The ability of hybrid dysgenesis *P* factors to survive and multiply under conditions of strong negative sterility selection was studied in mixed *P* and *M* laboratory cultures. Eight populations were initiated with varying proportions of *P* and *M* strains. Mixed populations and controls were maintained for seventeen generations at 27 °C, a temperature sufficiently high to induce maximum frequencies of sterility in dysgenic hybrids. The two components of dysgenesis, *P* factor activity and cytotype, were monitored every generation for the first ten generations and intermittently thereafter. With one exception, all the mixed populations evolved to the *P* type indicating that *P* factors can survive and multiply, despite low initial frequency and strong negative selection against dysgenic hybrids. However, the average level of *P* factor activity attained at equilibrium was considerably lower than that of the *P* strain control population maintained under the same conditions. It was also lower than the equilibrium level of *P* factor activity attained in a similar experiment carried out at a lower temperature, suggesting that selection favoured *P* factors with weak rather than strong sterility potential.

1. INTRODUCTION

The *P–M* system of hybrid dysgenesis and its associated syndrome of aberrant traits has been intensively studied at different levels of biological organization (for reviews see Bregliano & Kidwell, 1983; Engels, 1983). Dysgenic traits occur nonreciprocally in the hybrid progeny of *M* strain females mated with *P* strain males and include temperature-dependent sterility, chromosomal structural changes, male recombination, and increased frequencies of female recombination and lethal and visible mutation. These dysgenic traits are associated with the mobilization of a family of transposable genetic elements, referred to as *P* elements (Bingham, Kidwell & Rubin, 1982; O'Hare & Rubin, 1983).

P elements are mobilized in a maternal cytoplasmic state called *M* cytotype (Engels, 1979), which confers on the genome a susceptibility or lack of resistance to the action of such elements. They appear to be relatively stable in the cytoplasmic background of *P* strains (*P* cytotype). *P* elements vary in size from 2·9 to 0·5 kb and the smaller *P* elements have apparently arisen from the fully intact 'ancestral' 2·9 kb element by single internal deletions (O'Hare & Rubin, 1983).

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A fully functional *P* strain carries 30–50 *P* elements, approximately one-third of which are of the large (2·9 kb) size. Strains with limited *P* functions such as *Q* strains (Engels & Preston, 1981; Kidwell, 1979) probably have either a smaller total number of elements or a larger proportion of defective elements (Simmons *et al.* 1984). Most long-established laboratory *M* strains are completely lacking in *P* elements (Kidwell, Kidwell & Sved, 1977; Bingham *et al.* 1982) and their associated functions.

Population surveys of *P* and *M* strains have demonstrated major differences in the distribution of *P* elements among long-established laboratory and wild strains. Strains brought into the laboratory from the wild more than 30 years ago are almost always of the *M* type, while the majority of strains captured from American natural populations are of either the *P* or *Q* types (Kidwell, Frydryk & Novy, 1983). Two hypotheses have been proposed to explain this distribution. One, the 'recent invasion' hypothesis, suggests that *P* elements originated in the wild and invaded natural populations in the past ten to thirty years (Kidwell, 1979, 1983*a*). According to this hypothesis, strains brought into the laboratory from the wild over thirty years ago have been protected from invasion by *P* elements. The second, the laboratory loss hypothesis, suggests that *P* elements have historically been present in wild populations, but that they have been lost in long-established laboratory strains because of drift or changes in environment. Thus, strains brought into the laboratory over thirty years ago would have carried *P* elements at the time of capture, but would have lost them during subsequent culture. The stochastic loss hypothesis (Engels, 1981) proposes a mechanism by which recent loss may have occurred.

In order to begin to understand the population dynamics of these mobile elements and to attempt to distinguish between various hypotheses for their evolution, extensive studies of the fate of *P* elements in mixed population are needed. The purpose of this paper is to report the outcome of mixed population studies carried out under high temperature conditions which are expected to exert strong selection pressure against dysgenic hybrids and the *P* elements they carry.

2. MATERIALS AND METHODS

The following strains of *Drosophila melanogaster* were employed:

(*a*) Strains used for experimentation:
 Cranston-4, a moderately strong *P* strain.
 Tester-I, a strong *M* strain.

(*b*) Reference strains:
 Canton-S, a very strong *M* strain.
 Harwich, a very strong *P* strain.

See Kidwell, Novy & Feeley (1981) for further details of strains. Flies were raised on a standard cornmeal-agar-molasses-yeast medium, seeded with live yeast.

Method of monitoring populations for P and M characteristics

P factor activity was monitored by a standard diagnostic mating called Cross A: 20 Canton-S♀♀ × 20♂♂ of population under test. Cytotype was monitored by the standard Cross A* mating: 20♀♀ of population under test × Harwich♂♂. Cross

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A and Cross A* test matings were maintained during development at 29 °C which is a restrictive temperature for gonadal sterility. After eclosion, approximately fifty F_1 females from each mating were aged for two days and then dissected in water. The frequency of ovarian dysgenesis was estimated according to previously established criteria (Schaefer, Kidwell & Fausto-Sterling, 1979). The degree of ovarian dysgenesis in Cross A is indicative of the level of *P* factor activity. The degree of ovarian dysgenesis in Cross A* is an indicator of cytotype; high sterility indicates *M* cytotype and low sterility indicates *P* cytotype.

Table 1. *Strain source and numbers of parental flies used to initiate mixed populations*

Population number	Cranston-4 (*P*) ♀	Cranston-4 (*P*) ♂	Tester-I (*M*) ♀	Tester-I (*M*) ♂	% *P* flies
1	25	25	—	—	100
2	—	—	25	25	0
3	—	5	25	20	10
4	—	15	25	10	30
5	5	—	20	25	10
6	15	—	10	25	30
7	—	25	25	—	50
8	25	—	—	25	50

3. RESULTS

Six populations were initiated with varying proportions and maternal and paternal contributions from the Cranston-4 (*P*) and Tester-I (*M*) strains. Overall, there was an initial preferential bias towards the *M* type, as shown in Table 1. Populations 1 and 2 were *P* and *M* strain controls, respectively. All eight populations were maintained continuously at a temperature of 27 °C throughout the entire experiment. This temperature was chosen to insure the full expression of sterility in dysgenic hybrids (Kidwell & Novy, 1979) but to avoid the effects of high temperature due to other causes. The populations were maintained by bottle to bottle transfer and for the first ten generations they were monitored at each generation for *P* activity (diagnostic Cross A) and for *P* or *M* cytotype (diagnostic Cross A*). Thereafter, monitoring occurred at three to four generations intervals.

Figs. 1–4 show the evolution of *P* factor activity over 17 generations. For comparative purposes, in Figs. 1–3 the results for each set of populations which were initiated with the same proportion of *P* flies are shown together with those for the two control populations. Fig. 5 shows the evolution of cytotype for all populations together. The two mixed populations which were started with the lowest frequency of *P* flies differed in their *P–M* characteristics throughout the course of the experiment (Figs. 1 and 5). Population 5 (10% *P* contributed maternally) showed weak to moderate levels of *P* factor activity (approximately 25% gonadal sterility potential). This population rapidly evolved to *P* cytotype

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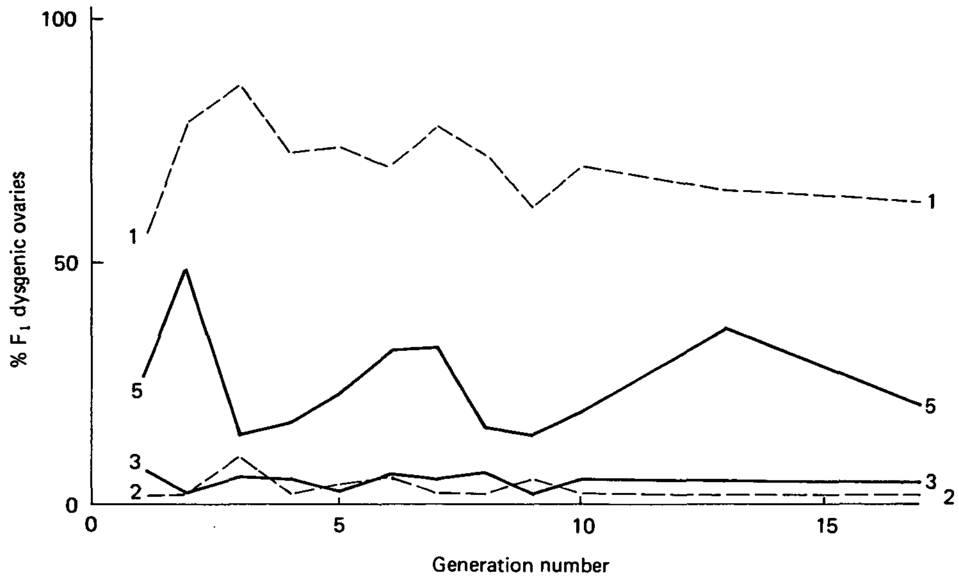

Fig. 1. Cross A sterility frequencies in samples from populations 3 and 5 (started with 10% *P* flies) and the control populations 1 and 2.

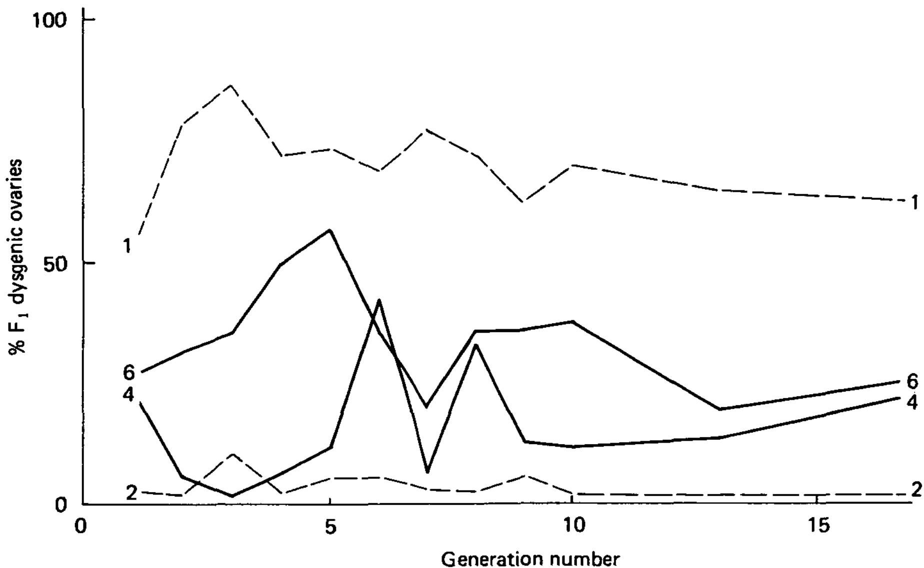

Fig. 2. Cross A sterility frequencies in samples from populations 4 and 6 (started with 30% *P* flies) and the control populations 1 and 2.

within 5 generations (Fig. 5). In contrast, population 3 (10% of *P* contributed paternally) gave no indication of *P* factor activity (Fig. 1) and the cytotype was similar to that of the *M* control population throughout the experiment.

The Cross A results for the two mixed populations started with 30% *P* are given in Fig. 2. The equilibrium level of *P* activity was weak to moderate for both

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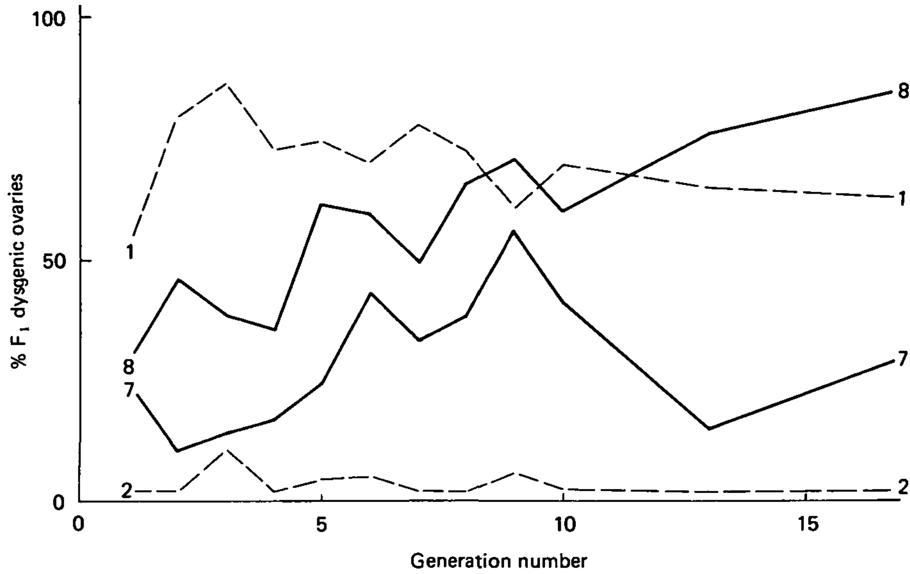

Fig. 3. Cross A sterility frequencies in samples from populations 7 and 8 (started with 50 % *P* flies) and the control populations 1 and 2.

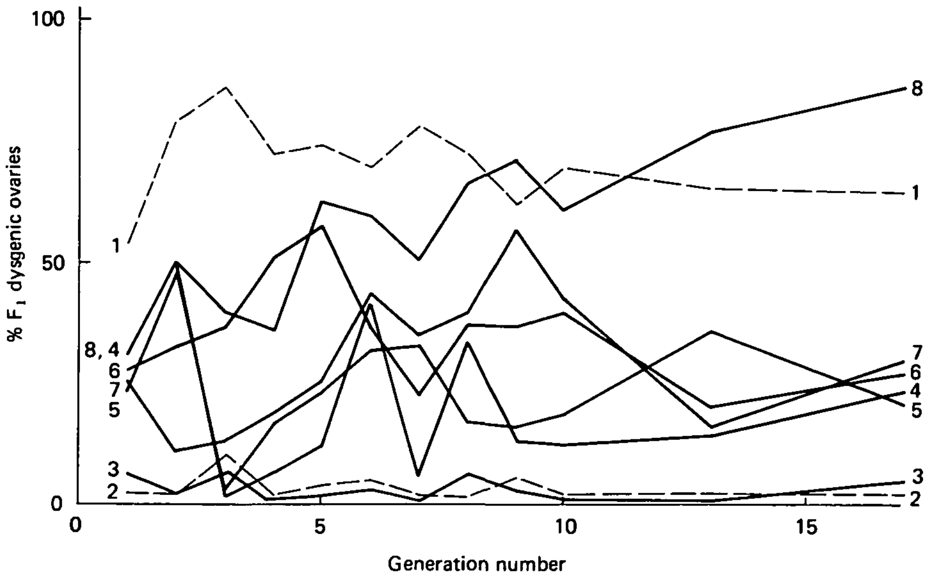

Fig. 4. Evolution of *P* factor activity. Summary of Cross A sterility frequencies in samples from all eight populations, including controls.

populations, but population 6 (*P* contributed maternally) fairly consistently had a higher sterility potential than population 4 (*P* contributed paternally). Fig. 5 indicates that both populations evolved to the *P* cytotype, population 6 very rapidly and population 4 relatively slowly.

Populations 7 and 8 were both started with equal proportions of *P* and *M* flies

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but in reciprocal parental combinations. The equilibrium level of *P* factor activity of population 7 (*P* contributed paternally) was approximately the same as that of populations 4, 5 and 6 (see Fig. 4). However, population 8 (*P* contributed maternally) showed a consistent and fairly linear increase in *P* factor activity throughout the course of the experiment. The cytotypes of both populations 7 and 8 very rapidly switched to the *P* type.

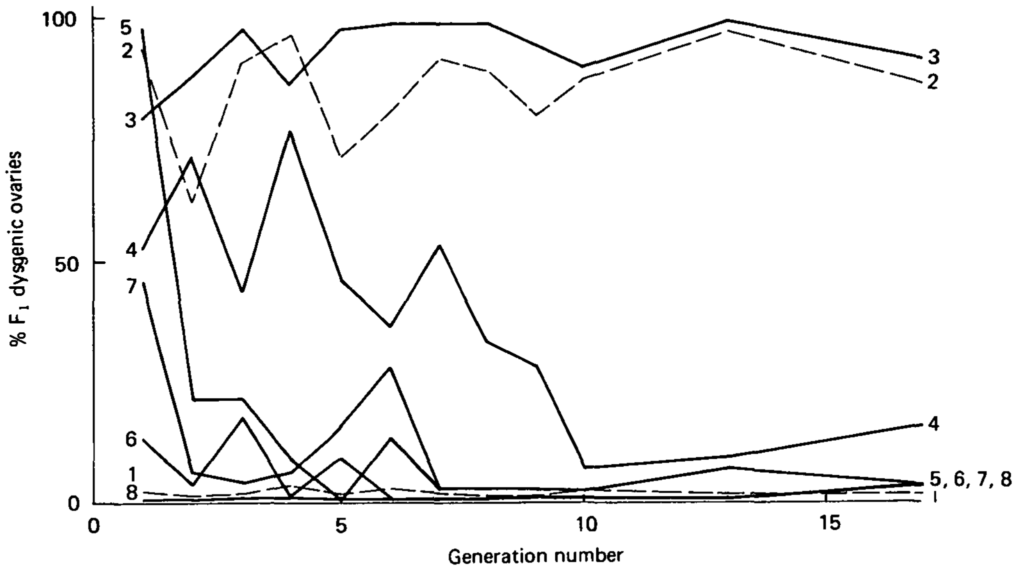

Fig. 5. Evolution of cytotype. Summary of Cross A* sterility frequencies in samples from all eight populations, including controls.

Overall, the Cross A data (Fig. 4) indicate that, with one exception, all mixed populations evolved to the *P* type. This conclusion is confirmed by the Cross A* data (Fig. 5). The exceptional population showed no phenotypic indication of the acquisition of *P* factors. The levels of *P* factor activity at equilibrium in the majority of mixed populations were weak to moderate and significantly lower than the level of the *P* strain control. Also, in those pairs of populations which were initiated with the same proportions of *P* flies, the paternally contributing population consistently had a lower level of *P* activity than the maternally contributing population.

4. DISCUSSION

The evolution of the majority of mixed populations to the *P* type suggests that the high temperature sterility potential of dysgenic hybrids did not, in most instances, provide a selective pressure great enough to eliminate *P* elements, even at low initial frequency. On the contrary, the processes favouring the multiplication of *P* factors were apparently sufficient to offset negative selection, leading to the evolution of relatively stable *P* strains within five to ten generations.

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These results confirm and extend those of an earlier experiment (Kidwell *et al.* 1981) in which the same strain combinations and a similar experimental design were used but in which the mixed populations were maintained continuously at 20 °C, a temperature at which no sterility selection is expected. The results of the two sets of experiments are qualitatively similar in that evolution to the *P* type generally occurred. Also, in both experiments, the level of *P* factor activity (Cross A) was unexpectedly high in the early generations but usually showed no consistent changes thereafter. However, there is a marked quantitative difference between the two temperature groups in the average level of *P*-factor-related sterility potential which is attained at equilibrium. The populations maintained at 20 °C (Kidwell *et al.* 1981) attained levels of sterility similar to those of the unmixed *P* strain controls. In contrast, the present results obtained at high temperature indicate that, with one exception, the equilibrium sterility potential was significantly lower than that of the *P* strain control. This observation suggests that high temperature maintenance conditions exert selection against those *P* elements that have the potential for a high sterility phenotype and favour those in which this potential is modified.

The *P* family of transposable elements is known to be highly heterogeneous in both structure and function (Bregliano & Kidwell, 1983; O'Hare & Rubin, 1983). Fully intact *P* elements, 2·9 kb in length and possessing all *P* strain functions, represented only one-third of the total number of *P* elements present in the strong *P* strain, π_2; two-thirds of the elements were shorter in length and an unknown fraction of these presumably had some type of functional deficiency. It is suggested that the conditions pertaining in the majority of mixed populations during the early stages of the present experiment (i.e. high temperature and a high frequency of *M* cytotype) allowed the *P* elements to manifest their functional potential at the phenotypic level of the fly. Thus, an opportunity was provided for natural selection to distinguish between varying levels of sterility associated with structurally and functionally different *P* elements. In a stable *P* strain, such as control no. 1, *P* elements are repressed by *P* cytotype and sterility does not occur, even at high temperature. There is thus no opportunity for selection to act on functional differences among the *P* elements present and those with strong sterility potential are maintained. This interpretation is strengthened by the observation in population no. 8 of an equilibrium sterility potential equal to that of the no. 1 control. It should be noted that no. 8 was the only mixed population which was initiated with 100% *P* cytotype. Thus, the $P \times M$ hybrids produced in the very early, critical generations would not be expected to show gonadal sterility. As in the control, there would, therefore, be little opportunity for selection to distinguish between functional differences among *P* elements.

There was one exceptional population (no. 3) that showed no indication of any degree of *P* factor activity or *P* cytotype. This population was initiated with 10% *P* flies, contributed paternally. In the first generation there were thus five *P* males in an otherwise *M* population. It is possible that not all five males succeeded in mating. Those that did succeed would be expected to produce high frequencies of sterile progeny. Thus, by a combination of chance and strong selection against dysgenic hybrids, it seems possible that none of the original five males contributed

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germ plasm to succeeding generations and the population behaved in an identical way to the *M* strain control.

Two not necessarily mutually exclusive mechanisms might account for the observed evolution of mixed populations to the *P* type. The first is due to the 'selfish DNA' characteristics of *P* elements resulting in an increase in *P* element copy number by transposition in *M* cytotype. Transposition of *P* elements *per se* is apparently not dependent on high developmental temperature (Bingham *et al.* 1982; Kidwell, 1983*b*), but its effects in increasing the number of *P* element copies would be opposed by high temperature sterility selection. Transposition is expected to be repressed when *P* cytotype has evolved and *P* element copy number and function become stabilized.

The second possible mechanism for evolution of the *P* type is that *P* strain chromosomes possess an intrinsic fitness advantage over *M* strain chromosomes unrelated to the ability of *P* elements to multiply by virtue of transposition. Such a fitness advantage might be totally unrelated to the presence of *P* elements or it might exist because of some *P* element property other than transposability. The steady increase in *P* factor activity in population no. 8 has strong similarities to an artificial selection curve and might provide evidence for the second type of mechanism. However, the behaviour of this population can also be explained by the first mechanism. The second mechanism alone seems less plausible for explaining evolution of the *P* type in the other mixed populations. A selective advantage of *P* over *M* chromosomes would have to be very strong to offset the strong negative effect of sterility selection.

The results of this and the earlier 20 °C experiment are consistent with the predictions of the rapid invasion hypothesis and provide strong support for an important assumption of this hypothesis: that *P* strains are able to rapidly invade predominantly *M* populations despite low initial frequency and strong selection against $M \times P$ hybrids. The present experiment may also provide some answers to the question of the reason for the wide quantitative variability of *P* factor function in natural populations. Temperature may exert a strong influence in determining the level of *P* factor activity (sterility) in newly invaded populations. Extreme high temperatures would be expected to exert strong selection for *P* elements with low sterility potential with the consequent evolution of *P* strains lacking sterility potential (*Q* strains). In contrast, lower mean temperatures in temperate climates would not be expected to exert such pressures and might lead to the survival of *P* elements with stronger sterility potential. In practice most strains will be expected to carry *P* elements with a wide range of sterility potential, resulting in a high degree of polymorphism for *P* factor activity. However, founder effects might be important in certain geographic areas resulting in the spread of either predominantly weaker or stronger elements with a consequent variability in geographical distribution.

This research was supported in part by the National Institute of General Medical Sciences (GM-25399).

https://doi.org/10.1017/S0016672300026495 Published online by Cambridge University Press

REFERENCES

BINGHAM, P. M., KIDWELL, M. G. & RUBIN, G. M. (1982). The molecular basis of *P–M* hybrid dysgenesis: the role of the *P* element, a *P*-strain-specific transposon family. *Cell* **29**, 995–1004.

BREGLIANO, J. C. & KIDWELL, M. G. (1983). Hybrid dysgenesis determinants. In *Mobile Genetic Elements* (ed. J. A. Shapiro), pp. 363–410. New York: Academic Press.

ENGELS, W. R. (1979). Hybrid dysgenesis in *Drosophila melanogaster*: rules of inheritance of female sterility. *Genetical Research* **33**, 219–236.

ENGELS, W. R. (1981). Hybrid dysgenesis in *Drosophila* and the stochastic loss hypothesis. *Cold Spring Harbor Symposium of Quantitative Biology* **45**, 561–565.

ENGELS, W. R. (1983). The *P* family of transposable elements in *Drosophila*. *Annual Review of Genetics* **17**, 315–344.

ENGELS, W. R. & PRESTON, C. R. (1981). Characteristics of a neutral strain in the *P–M* system of hybrid dysgenesis. *Drosophila Information Service* **56**, 35–37.

O'HARE, K. & RUBIN, G. M. (1983). Structures of *P* transposable elements of *Drosophila melanogaster* and their sites of insertion and excision. *Cell* **34**, 25–35.

KIDWELL, M. G. (1979). Hybrid dysgenesis in *Drosophila melanogaster*: the relationship between the *P–M* and *I–R* interaction system. *Genetical Research* **33**, 205–217.

KIDWELL, M. G. (1983*a*). Evolution of hybrid dysgenesis determinants in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences* (*U.S.A.*) **80**, 1655–1659.

KIDWELL, M. G. (1983*b*). Hybrid dysgenesis in *Drosophila melanogaster*: factors affecting chromosomal contamination in the *P–M* system. *Genetics* **104**, 317–341.

KIDWELL, M. G. (1979). Hybrid dysgenesis in *Drosophila melanogaster*: sterility resulting from gonadal dysgenesis in the *P–M* system. *Genetics* **92**, 1127–1140.

KIDWELL, M. G. & NOVY, J. B. (1979). Hybrid dysgenesis in *Drosophila melanogaster*: Sterility resulting from gonadal dysgenesis in the P–M system. *Genetics* **92**, 1127–1140.

KIDWELL, M. G., KIDWELL, J. F. & SVED, J. A. (1977). Hybrid dysgenesis in *Drosophila melanogaster*: a syndrome of aberrant traits including mutation, sterility and male recombination. *Genetics* **86**, 813–833.

KIDWELL, M. G., NOVY, J. B. & FEELEY, S. M. (1981). Rapid unidirectional change of hybrid dysgenesis potential in *Drosophila*. *Journal of Heredity* **72**, 32–38.

SCHAEFER, R. E., KIDWELL, M. G. & FAUSTO-STERLING, A. (1979). Hybrid dysgenesis in *Drosophila melanogaster*: morphological and cytological studies of ovarian dysgenesis. *Genetics* **92**, 1141–1152.

SIMMONS, M. J., RAYMOND, J. D., JOHNSON, N. A. & FAHEY, T. M. (1984). A comparison of mutation rates for specific loci and chromosome regions in dysgenic hybrid males of *Drosophila melanogaster*. *Genetics* **106**, 85–94.

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