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Hybrid dysgenesis in *Drosophila melanogaster*: synthesis of *RP* strains by chromosomal contamination

MARGARET G. KIDWELL*
Division of Biology and Medicine, Brown University, Providence, Rhode Island 02912 U.S.A.

AND HELEN M. SANG†
Department of Molecular Biology, University of Edinburgh, Kings Buildings, Edinburgh EH9 3JR, Scotland

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Summary

Several strains have been synthesized which have reactive (*R*) properties in the *I–R* system of hybrid dysgenesis and which are also classified as active *P* strains in the *P–M* system. The synthesis of this previously unknown combination of types was accomplished by employing a mating scheme which allowed transposition (chromosomal contamination) of *P*, but not *I*, factors from *IP* to *RM* chromosomes in dysgenic F_1 males. The successful synthesis of *RP* strains provides strong evidence that the apparent absence of this combination in natural and laboratory populations of *melanogaster* is not due to a biological incompatibility between these two types.

1. Introduction

Two systems of hybrid dysgenesis in *D. melanogaster* (the *P–M* and *I–R* systems) were initially recognized on the basis of their phenotypic properties and patterns of inheritance (Kidwell, Kidwell & Sved, 1977; Picard, 1976). Hybrid dysgenesis is manifested in the form of a number of correlated aberrant genetic traits such as high frequencies of sterility, male recombination and mutation (see Bregliano & Kidwell, 1983, for a review of the two systems). The specific sets of traits associated with each system are similar in general properties but they differ in detailed characteristics. One of the most prominent differences is that dysgenic traits are limited to female hybrids in the *I–R* system but occur in both sexes in the *P–M* system. In both systems, the genetic events leading to dysgenesis are almost always restricted to the germ line and to one of the two classes of hybrids produced by reciprocal crosses between interacting strains.

Every strain or population of *melanogaster* may be characterized with respect to its potential within each of the two systems of hybrid dysgenesis. In the *P–M* system, two main classes of strains, *P* and *M*, have been described on the basis of their functional properties (Kidwell *et al.* 1977). Hybrids between *P* strain males and *M* strain females show significant frequencies of gonadal sterility, male recombination and other dysgenic traits. A subset of *P* strains known as *Q* strains do not have a potential for gonadal sterility in any strain combination but produce low frequencies of other dysgenic traits in crosses with *M* strain females.

At the molecular level, a family of mobile elements, the *P* elements, has been shown to be responsible for the manifestations of *P–M* hybrid dysgenesis (Rubin, Kidwell & Bingham, 1982). They found that *P* and *Q* strains carry 30–50 *P* element copies per haploid genome. Both *P* and *Q* classes exhibit a cellular property called *P* cytotype which confers resistance to the disruptive activities of *P* elements. *M* strains have no potential for *P* factor activity but they possess an *M* cytotype which confers susceptibility to the action of *P* elements (Engels, 1979). *M* strains are of two main types. True *M* strains, completely lack *P* elements and have extreme *M* cytotype; they are well represented amongst long established laboratory strains (e.g. Bingham, Kidwell & Rubin, 1982). In contrast, pseudo-*M* (*M′*) strains carry *P* elements, many or all of which are defective. The ability of *M′* strains to suppress *P* element transposition may show a continuous range of variability (Kidwell, 1985).

In the *I–R* system, two main classes of strains have been recognized, inducer (*I*) and reactive (*R*). A third

* Present address: Department of Ecology & Evolutionary Biology, University of Arizona, Tucson, AZ 85721, U.S.A.
† Present address: Poultry Research Centre, Roslin, Midlothian EH25 9PS Scotland

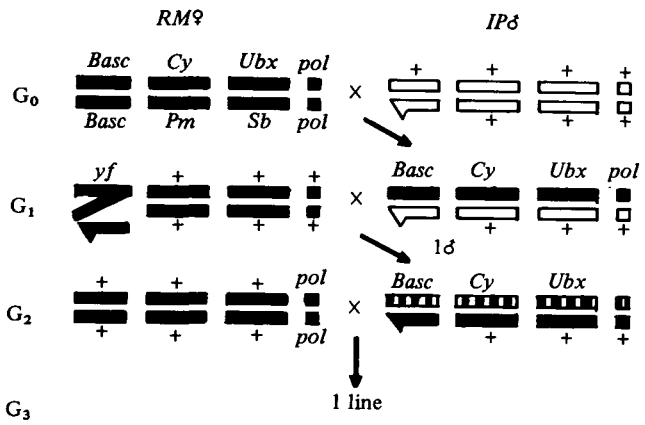

Fig. 1. Mating scheme used to synthesize *RP* lines. Solid, open and hatched bars denote *RM*, *IP* and putative *RP* chromosomes respectively.

class, referred to as neutral (*N*), appears to be a weak subset of the *R* class (Bregliano *et al.* 1980). Female hybrids produced by matings between *I* males and *R* females show *SF* sterility, increased mutation rates and other dysgenic traits. *N* strains do not produce significant frequencies of hybrid dysgenesis in crosses with either inducer or reactive strains. A mobile element called the *I* factor has been shown to be responsible for *I–R* hybrid dysgenesis (Bucheton *et al.* 1984). Active *I* factors are apparently only present in inducer strains in approximately 15 copies per haploid genome. However, non functional *I* element sequences are present in a stable state in *I*, *R* and *N* strains.

Extensive surveys of both natural and laboratory populations have shown that, of the four possible combinations of *P*, *M*, *I* and *R* types, three, *IP*, *IM* and *RM*, are well represented. However, the existence of the fourth, *RP*, type has not previously been reported. The absence of this type has been attributed variously to an outcome of the evolutionary history of the two systems or to some form of biological constraint in which *P* elements are incompatible with a reactive state (Kidwell, 1979, 1983).

The purpose of this paper is to report the successful synthesis of an *RP* strain using an experimental design which makes use of the different transposition properties of *I* and *P* elements in male dysgenic hybrids.

2. Materials and Methods

Strains employed. For details of mutants and balancers, see Lindsley & Grell (1968). (The two letters in parenthesis after the name of each strain refer to the designation of the strain in the *I–R* and *P–M* systems of hybrid dysgenesis respectively.)

(1) H-41 (*RM*): *Basc*, $w^a B$; *In(2LR)*bw^{V1}, ds^{33k} *dp b* bw^{V1}/*SM1*, $al^2 Cy$ $cn^2 sp^2$; *Sb/TM2*, UBx^{130} e^s; spa^{pol}. A multiple chromosome balancer strain (Kidwell, 1985) completely lacking *P* element homology (Bingham *et al.* 1982).

(2) Harwich (*IP*): A strong *P* strain used as a reference strain for the *P–M* system (Kidwell *et al.* 1977).

(3) *C(1)DX, yf/+* (*RM*): A long-established attached-X laboratory strain.

(4) spa^{pol} (*NM*): A laboratory strain homozygous for the chromosome 4 mutant sparkling-poliert which was derived from the H–41 strain described above.

(5) Cockaponsett Forest (*RM*): A long established wild-type laboratory strain used as a reference strain for the *I–R* system. (Kidwell, Frydryk & Novy, 1983).

(6) Canton S (*IM*): A long established wild-type laboratory strain. This strain carries no *P* elements and was used as an *M* reference strain in the *P–M* system and an *I* reference strain in the *I–R* system. (Kidwell *et al.* 1977).

(7) Ashwood (*IM*): A laboratory wild-type strain originating in a natural population collected in England prior to 1970 (Kidwell *et al.* 1983). Because of its strong inducer properties, it is now used as a reference *I* strain for the *I–R* system in the laboratory of one of us (M.G.K.).

Mating scheme for construction of RP *stock*

A mating scheme was designed to synthesize strains of flies having *RP* characteristics. It employed three generations of matings as shown in Fig. 1. The strategy was to produce F_1 hybrid males from a cross between *RM* females carrying marked balancer chromosomes and males from a wild-type *IP* strain. Although such F_1 hybrid males are produced from a cross that is potentially dysgenic for both the *I–R* and *P–M* systems of hybrid dysgenesis, transposition of *P* elements but not *I* elements from *IP* to *RM* chromosomes would be expected to occur because there is no evidence that *I* elements are mobilized in males (Picard, 1976). In the G_2 generation, individual males were selected which received marked balancer first, second, and third chromosomes from their fathers and the homologues from their *M* (attached-*X*) mothers. Thus, after a single generation of dysgenesis, all the original, Harwich-derived, *P* chromosomes were removed. In order to ensure that the *P* strain chromosome 4 was also not present, G_2 males were progeny tested for the presence of spa^{pol} (see Fig. 1). Only those lines were retained for future testing which derived from G_2 males and had received a spa^{pol} (*M*) rather than a *P*, fourth chromosome from their F_1 fathers. From G_3 onwards the lines were maintained by mass mating.

Phenotypic monitoring of P–M *and* I–R *strain characteristics*

In order to test for the characteristics of each system of hybrid dysgenesis, two crosses, were made *en masse* between the reference strain and the strain under test as shown in Table 1. The frequency of F_1 ovarian dysgenesis (Schaefer, Kidwell & Fausto-Sterling,

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Table 1. *Standard mating schemes to test a strain of unknown characteristics (strain U) for properties of the two systems of hybrid dysgenesis*

System (temp.)	Gen. no.	Cross A: test for *P* or *I* activity Females	Males	Cross A*: test for cytotype Females	Males
P–M (29°)	G_0	Canton-S(*IM*) × Strain U		Strain U × Harwich(*IP*)	
		↓		↓	
	F_1	Score *GD* sterility		Score *GD* sterility	
I–R (20°)	G_0	Gruta (*RM*) × Strain U		Strain U × Ashwood (*IM*)	
		↓		↓	
	F_1	Mate *inter se*		Mate *inter se*	
		↓		↓	
	G_2	Score *SF* sterility (egg hatchability)		Score *SF* sterility (egg hatchability)	

Table 2. *Percentage of F_1 ovarian dysgenesis* (GD *sterility) from crosses A and A* at selected generations. Approximately* 50 *F_1 hybrid females were examined per cross.*

	Generation 14		Generation 30	
Line number	A	A*	A	A*
3	91	0	75	1
4	4	91	0	99
9	97	66	92	1
23	89	2	99	0
30	7	77	1	90
35	13	14	3	4
43	2	100	0	83
48	76	0	46	3

1979; Engels & Preston, 1979) was used to assay for the *P–M* system and the frequency of *SF* sterility (Picard, 1976) was used to assay for the *I–R* system. Canton-S or Ashwood replaced Luminy as the inducer reference strain (see Kidwell, 1984, for rationale and further details of tests).

DNA preparation, gel electrophoresis and Southern transfer experiments

Drosophila DNA preparation, gel electrophoresis, filter preparation and hybridization were carried out as described in Bucheton *et al.* (1984). The complete *P* factor clone, pπ25·1 (O'Hare & Rubin, 1983), was used to detect *P* sequences. The plasmid pI59 (Bucheton *et al.* 1984), containing the 2·3 kb internal Hind III/Pst I fragment from an *I* factor, was used to identify *I* factors.

3. Results

With the object of making a number of independent *RP* lines, crosses were set up as shown in Fig. 1 and as described in Section 2. Forty-eight lines, each derived from a single G_2 male were progeny tested for an *M*-derived fourth chromosome marked with spa^{pol}. Thirteen of these lines were carried forward for further testing.

Phenotypic monitoring: P–M *system*

Using gonadal sterility as the method of assay, *P* factor activity was measured in Cross A and cytotype in Cross A* (see Table 1 for details of mating schemes). Fifty F_1 females were dissected from each line and generation tested.

In the first generation of testing, five out of thirteen lines showed no sterility in F_1 females from cross A matings and were discarded. The remaining eight lines were continued and tested at intervals over 30 generations. The results of the Cross A and A* tests for generations 14 and 30 are shown in Table 2. A detailed analysis of the dynamics of *P* factor activity and cytotype in these eight lines will be reported elsewhere (M. G. Kidwell, in preparation).

All eight of the tested lines showed an increase in gonadal sterility over the first four to six generations. However, as seen in Table 2, after 30 generations, there were large differences in the outcome of the evolution of the *P* element family in these eight lines. Half of the eight lines (numbers 3, 9, 23 and 48) evolved to strong *P* strains with a clearly defined *P* cytotype. One line, number 35, evolved to a weak *P* (*Q*) strain with *P* cytotype. Three lines (numbers 4, 30 and 43) lost their original potential for producing *GD* sterility in Cross A and retained an *M* cytotype.

Phenotypic monitoring: I–R *system*

Standard phenotypic tests for *I* factor activity and reactivity were carried out at generation 6 using *SF* sterility as the method of assay (see Table 1). The

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Table 3. *Percentage of unhatched eggs* (±S.E.) *from crosses A and A* at generation 6. Between 100 and 200 eggs, laid by the three groups of* F_1 *females, were examined per cross.*

	Cross	
Line number	A	A*
3	6·2±2·1	41·6±6·5
4	4·0±1·4	9·3±1·8
9	3·0±0·2	17·0±4·1
23	0·9±0·5	21·4±3·1
30	15·7±2·7	13·1±4·6
35	11·0±1·9	17·4±5·3
43	6·1±1·3	11·5±2·3
48	23·3±1·5	41·0±5·2

Table 4. *Percentage of unhatched eggs* (±S.E.) *from crosses A and A* involving selected sublines of RP48–20 tested at generation 30. Between 200 and 300 eggs laid by three sets of* F_1 *females were examined per cross.*

	Cross	
Line number	A	A*
1	30±1·6	81±0·3
2	41±3·2	45±1·3
3	36±1·9	48±2·2
4	ND	63±7·8
5	33±3·5	41±2·4
6	33±1·2	55±6·3
7	34±1·8	74±0·9
8	38±2·9	63±4·6
9	38±5·0	75±2·6
10	32±2·4	65±4·5
11	40±2·7	42±3·4
12	30±1·5	46±2·0
Control	99±1·3	100±0·3

ND, not determined.

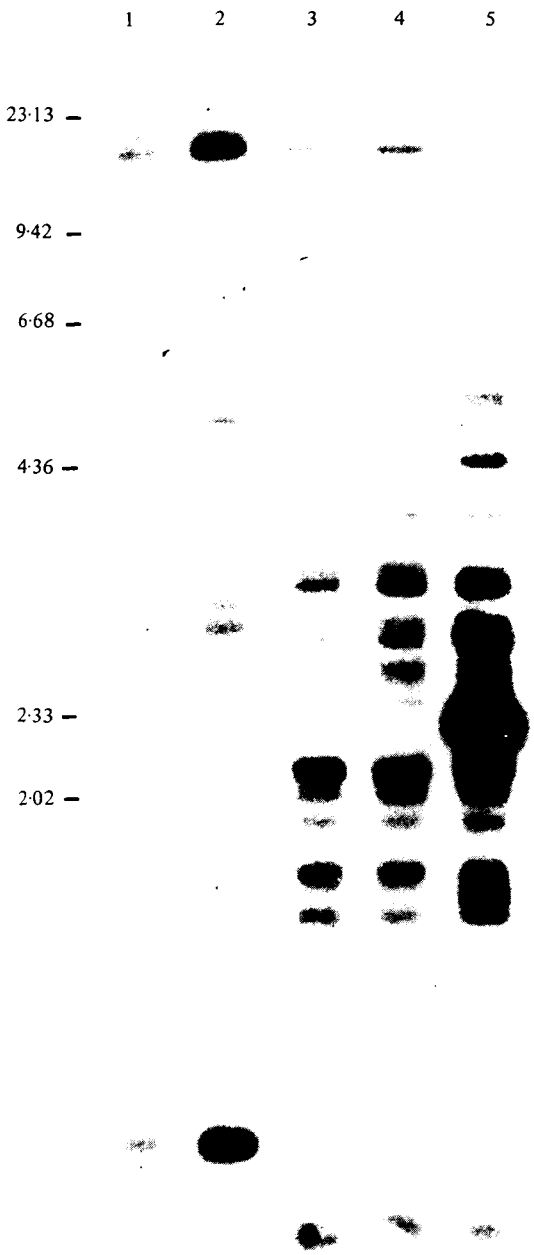

Fig. 2. Autoradiograph of Southern transfer experiment showing hybridization of *P* and *I* factor sequences RP48-7 and RP48-20. Lanes 1 and 2 show genomic DNAs from RP48-7 and RP48-20 digested with Hind III, run on a 1% agarose gel, transferred to nitrocellulose, and probed with the *P* factor clone, pπ25·1. Lanes 3, 4 and 5 contain genomic DNAs from RP48-7, RP48-20 and Bojo, an *I* strain, digested with Hind III and Pst *I*, transferred to nitrocellulose and probed with p159, the subcloned 2·3 kb Hind III/Pst I internal fragment from an *I* factor.

results are presented in Table 3. The frequency of sterility in cross A* was in every instance equal to or greater than that in cross A. On the basis of these phenotypic results, these lines were classified as neutral or weakly reactive during the early generations of the experiment.

The line with the highest level of reactivity, no. 48, was divided into a number of sublines. Two of these, 48-7 and 48-20, which had the highest reactivity, were subjected to another generation of selection and maintained in large mass matings for further testing. Line 48-20 was again subdivided and twelve subsublines were subjected to phenotypic tests at generation 30. The results are presented in Table 4.

In the hybrids from Cross A (*RM* × putative *RP*), the moderate values of unhatched eggs may be reasonably ascribed to *EL* sterility rather than *SF* sterility (see Kidwell, 1984). Those from Cross A* (putative *RP* × *IM*) may be ascribed to *SF* sterility. Because both *SF* and *EL* types of sterility result in embryo death at late cleavage, they are not readily distinguishable from one another at the phenotypic level.

Molecular analysis

All eight lines were examined at generation 30 for the presence of *P* factor sequences. Using Southern blot

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analysis, all showed homology to an internal fragment of the *P* factor (M. G. Kidwell, unpublished results). The two sublines RP48–7 and RP48–20 were tested for homology to *P* and for the presence of *I* factors. Fig. 2 shows an autoradiograph of Hind III digests of DNA from RP48–7 and RP48–20 which were probed with a radioactively labelled *P* factor clone. Both strains have many hybridizing fragments. Fig. 2 also shows Hind III/Pst I digests of the two sublines and of Bojo, an *I* strain, probed with the internal 2·3 kb Hind III/Pst I fragment from an *I* factor. The presence of many copies of this fragment is diagnostic of an *I* strain (Bucheton *et al.* 1984). The Bojo strain shows a strongly hybridizing 2·3 kb fragment while the *RP* strains have one or no copies. The results of the molecular analysis of RP48–7 and RP48–20 confirm the conclusion of the phenotypic analysis that they are *RP* strains.

4. Discussion

The results of both phenotypic and molecular tests of *P–M* and *I–R* system characteristics, taken together, provide evidence for the successful synthesis of an *RP* strain. There appeared to be a little difficulty in generating a number of strong *P* strains providing that sufficient time was allowed for the evolution of strong *P* activity and the switch to *P* cytotype which stabilized the system. The weak reactivity of most of the synthesized strains can be explained by the cross to *N* (weak *R* females) in the G_2 generation (see Fig. 1) which was carried out in order to progeny test for the presence of the fourth chromosome from the original *RM* stock. The success of the subsequent selection procedure for strong reactivity was consistent with previous results with this character (Bucheton & Picard, 1978). Confirmation that the synthesis of the *RP* combination is possible has been provided by D. Anxolabéhère and his colleagues (personal communication) using the method of *P* element transformation.

Two types of explanation have previously been proposed to explain the observed absence of *RP* strains in natural and laboratory populations. One explanation has been that a biological constraint or incompatibility exists between *P* elements and the reactive maternal state, despite the apparent independence of the *P–M* and *I–R* systems in other respects. The results reported here argue strongly against this explanation. An alternative hypothesis is that the absence of the *RP* combination can be explained by the sequential invasion of natural populations by first, active *I* factors and later *P* elements. A requirement for this scenario would be that the invasion of natural populations by active *I* factors was complete before the arrival and spread of *P* elements in this species (Kidwell, 1983).

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