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The Molecular Basis of P–M Hybrid Dysgenesis: The Role of the P Element, a P-Strain-Specific Transposon Family

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Summary

We have shown previously that four of five *white* mutant alleles arising in P–M dysgenic hybrids result from the insertion of strongly homologous DNA sequence elements. We have named these P elements. We report that P elements are present in 30–50 copies per haploid genome in all P strains examined and apparently are missing entirely from all M strains examined, with one exception. Furthermore, members of the P family apparently transpose frequently in P–M dysgenic hybrids; chromosomes descendant from P–M dysgenic hybrids frequently show newly acquired P elements. Finally, the strain-specific breakpoint hotspots for the rearrangement of the π_2 P X chromsome occurring in P–M dysgenic hybrids are apparently sites of residence of P elements. These observations strongly support the P factor hypothesis for the mechanistic basis of P–M hybrid dysgenesis.

Introduction

The germline of an individual who is the offspring of a female of any of a number of strains of D. melanogaster classified as M strains crossed to a male of any of a number of strains classified as P strains shows several properties distinguishing it from the germline of an individual who is the offspring of a cross of an M male to an M female, a P male to a P female or an M male to a P female. These properties include substantially elevated rates of mutation, chromosomal rearrangement and illicit recombination in males. This set of effects is referred to, collectively, as the P–M hybrid dysgenesis syndrome (Kidwell et al., 1977; Sved, 1979). The genetic elements (P factors) responsible for the M strain–P strain distinction map to all of the major chromosomes (Engels, 1979a; M. G. Kidwell, unpublished observations); furthermore, the capacity to behave as a P chromosome can be acquired by an M chromosome as a result of passage through a P–M dysgenic hybrid (M. G. Kidwell, submitted manuscript). These observations, together with the biochemical analysis of dispersed, repeated, transposable DNA sequence elements in Drosophila (see Spradling and Rubin, 1981, for a review), led to the formulation of the P factor hypothesis for the mechanistic basis of P–M hybrid dysgenesis (Engels, 1981).

The P factor hypothesis proposes that the P–M hybrid dysgenesis syndrome results from the behavior of a dispersed, repeated, transposable DNA sequence element family (the P factor family) present in P strains and missing from M strains. The P factor hypothesis further supposes that the expression of the capacity to transpose (and possibly of other genetic elements) is activated in the progeny of males carrying the transposon family in question (P males) crossed to females lacking the transposon family (M females). The construct used to describe this phenomenon makes use of the term cytotype (Engels, 1979b). In particular, the P factor is supposed to be activated in the M cytotype and quiescent in the P cytotype. Furthermore, cytotype is supposed to be under the ultimate control of the chromosomal genotype (P chromosomes produce a P cytotype, and M chromosomes an M cytotype), but this control is presumed to require several fly generations to assert itself. A change in cytotype produced by a change in chromosomal genotype is referred to as cytotype conversion. Lastly, an individual who is the offspring of a cross of a member of a stable P strain to a member of a stable M strain is presumed to have the cytotype of its female parent. In terms of this descriptive construct, the progeny of P males crossed to M females carry the P factor transposon family from the male parent and the M cytotype from the female parent, and are thus dysgenic (the P factor is activated). The progeny of the reciprocal cross carry the P factor transposon family from the female parent but possess the P cytotype, also from the female parent, and are thus not dysgenic (the P factor is quiescent).

Our objective in the studies described here and in the accompanying paper (Rubin et al., 1982) has been to test several of the central predictions of the P factor hypothesis. Among the readily testable predictions of this hypothesis is the proposal that most or all mutant alleles arising in P–M dysgenic hybrids will result from the insertion of members of a single transposon family. We have shown previously that four of five *white* mutant alleles arising in P–M dysgenic hybrids result from the insertion of strongly homologous DNA sequences, and thus that this first prediction of the P factor hypothesis is apparently fulfilled (Rubin et al., 1982).

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DNA Sequence Insertions (P Elements) Responsible for *white* Mutant Alleles Arising in P–M Dysgenic Hybrids Are Homologous to a P-Strain-Specific, Dispersed, Repeated DNA Sequence Element Family

The P factor hypothesis predicts that the DNA sequence element insertions producing *white* mutant alleles arising in P–M dysgenic hybrids ($w^{\#6}$, $w^{\#12}$, $w^{hd80k17}$ and w^{hd81b9}; Rubin et al., 1982) should be members of a dispersed, repeated DNA sequence element family (the P element family) present in P strains and missing from M strains. Figure 1 shows the result of probing duplicate Southern filters with a 1.1 kb Xba I–Sac I fragment from the wild-type *white* locus region of the π_2 P strain (left panel) and the corresponding Xba I–Sac I fragment from the $w^{\#12}$ mutant allele (right panel) containing a ~1.2 kb P element insertion. (See Rubin et al., 1982, for a description of the detailed structures of the cloned segments from which these probes were excised.) We interpret the results of this experiment as follows. First, the probe fragment from the π_2 w^+ allele (left panel) shows significant homology to only a single restriction fragment from each of the strains examined (the presence of two restriction fragments in the Muller-5 strain is attributable to an insertion on the Xba I–Sac I interval as will be discussed below), and the sequences homologous to this fragment have been shown previously to be present in approximately one copy per haploid genome (Bingham et al., 1981; Levis, et al., 1982). Second, these same sequences, now containing the P element insertion responsible for the $w^{\#12}$ mutant allele (right panel), are homologous to approximately 30 restriction fragments in each P strain examined (Harwich, π_2, Cranston, Weymouth 74g, Madison-75, Mt. Carmel and ν_6) and in the Muller-5 (Birmingham) M strain, but are homologous to only the single fragment showing homology to the corresponding fragment from the π_2 w^+ allele in all other M strains examined (H40, *Sb/Ubx,* Canton-S, Cockaponsett Forest, NB-1 and Ives). Collectively, these observations demonstrate that the $w^{\#12}$ P ele-

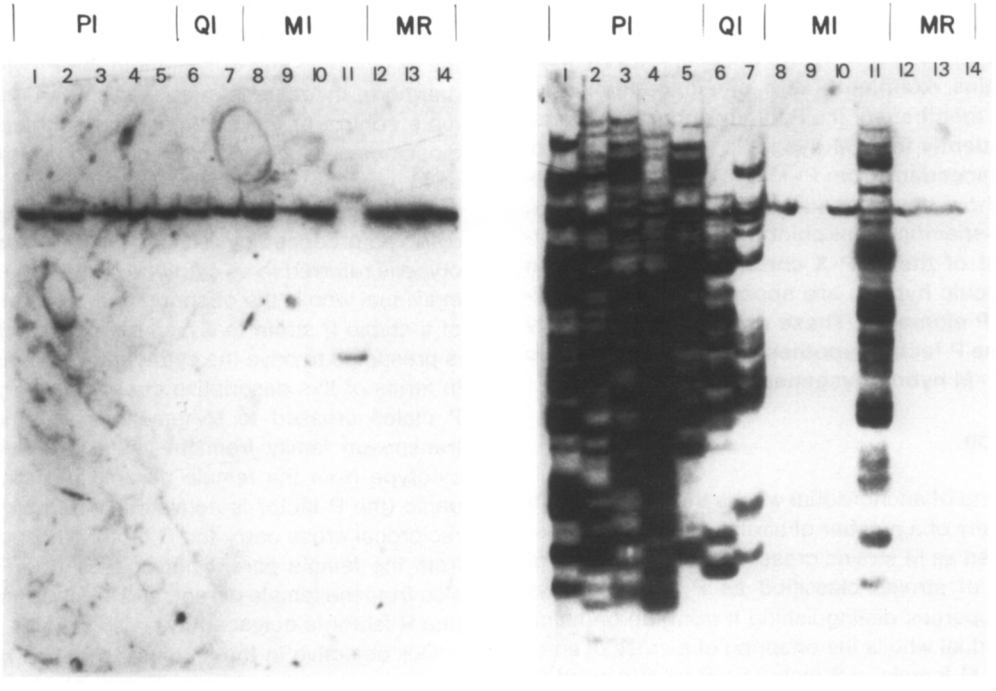

Figure 1. The P Element Is Homologous to P-Strain-Specific Repeated DNA Sequences

The figure shows duplicate Southern filters. The left-hand filter was probed with a unique *white* region restriction fragment (the 1.1 kb Xba I–Sac I fragment at coordinates −0.4 to +0.6 from the π_2 w^+ allele), and the right-hand filter was probed with the corresponding fragment carrrying the P element insertion (1.5 kb) responsible for the $w^{\#12}$ mutant allele at *white*. (Rubin et al., 1982, for a detailed description of the cloned segments from which these probes were excised.) Each of the 14 Drosophila DNAs was digested with Eco RI and Bam HI, which produces a 7.2 kb fragment from most w^+ alleles with homology to the unique sequences on the sequence probes; the single hybridizing fragment shown by most of the DNA samples in the left-hand panel is 7.2 kb. The Muller-5 (Birmingham) strain carries the w^a allele at *white*, and the two hybridizing restriction fragments in the Muller-5 lane (11) in the left-hand panel are those expected as a result of the presence of the *copia* transposon in this portion of the *white* locus region in the case of the w^a allele (Bingham et al., 1981; Bingham and Judd, 1981). P, Q and M refer to the classification of the Drosophila strains from which the DNAs were isolated with respect to the P–M hybrid dysgenesis system, and I and R refer to the classification of these strains with respect to the I–R system of hybrid dysgenesis (for example, a PI strain is P in the P–M system and I in the I–R system). Q refers to neutral strains in the P–M system; these strains are members of a special class of P strains, differing from standard P strains in only minor respects (Discussion; Kidwell, 1979; Engels and Preston, 1981b). The strains from which the Drosophila DNAs were isolated are as follows: π_2 (lane 1); Weymouth 74g (lane 2); Madison-75 (lane 3); Harwich (lane 4); Cranston (lane 5); Mt. Carmel (lane 6); ν_6 (lane 7); H-40 (lane 8); *Sb/Ubx* (lane 9); Canton-S (lane 10); Muller-5 (Birmingham) (lane 11); Cockaponsett Forest (lane 12); NB-1 (lane 13); Ives (lane 14).

ment insertion is homologous to repeated DNA sequences present in all P strains examined and missing from all M strains examined, with the exception of the Muller-5 (Birmingham) strain. Comparable results were obtained when the fragments containing the $w^{\#6}$, $w^{hd80k17}$ and w^{hd81b9} P element insertions were used as probes in analogous experiments (results not shown).

The Muller-5 (Birmingham) strain used in these experiments has been previously classified as an M strain, but, in contrast to the other M strains examined, it carries repeated DNA sequences homologous to the $w^{\#6}$, $w^{\#12}$, $w^{hd80k17}$ and w^{hd81b9} P element insertions. The question arises, therefore, whether the Muller-5 (Birmingham) strain was misclassified as M or the Muller-5 DNA sample was misidentified. We have confirmed that the Muller-5 (Birmingham) strain in our possession behaves as an M strain and not as a P strain by tests for gonadal sterility and male recombination in reciprocal crosses with standard P and M strains (see Kidwell, 1979, for a description of these tests). Furthermore, the strain in question carries the Muller-5 balancer X chromosome (Lindsley and Grell, 1968), which in turn carries the w^a allele at *white*. The w^a allele has been previously shown to be associated with the insertion of a copy of the *copia* transposable element into *white* locus DNA sequences (Bingham et al., 1981; Bingham and Judd, 1981). Southern gel analysis of the Muller-5 DNA sample used in these experiments shows the sample to carry an allele at *white* indistinguishable from the previously characterized w^a allele (left panel in Figure 1 and unpublished results). These observations demonstrate that in contrast to the other M strains examined in this experiment, the Muller-5 (Birmingham) strain carries repeated sequences homologous to P elements.

The P factor hypothesis further predicts that the repeated sequences homologous to P elements will be dispersed, residing on all the major chromosome arms. Figure 2 shows the result of an in situ hybridization experiment in which the DNA sequence probe is the 2.75 kb Sal I fragment from the $w^{\#6}$ allele consisting of 1.55 kb of unique *white* region DNA sequences and the 1.2 kb P element insertion responsible for the $w^{\#6}$ mutation (Rubin et al., 1982). The polytene salivary gland chromosomes were isolated from an individual from the π_2 P strain. The P element probe is homologous to approximately 30 euchromatic sites in the π_2 strain, and these sites are distributed among all the major chromosome arms (Figure 2), as predicted by the P factor hypothesis. All P strains examined (see legend to Figure 1 for a list) likewise show dispersed sites of residence of the P element (results not shown).

Breakpoint Hotspots for P Chromosome Rearrangement Occurring in P–M Dysgenic Hybrids Are Apparently Sites of Residence of the P Element

Berg et al. (1980) and Engels and Preston (1981a) observed that X chromosomes of the π_2 P strain frequently undergo gross rearrangement in the germline of P–M dysgenic hybrids. The breakpoints for these aberrations were not randomly distributed but rather almost always occurred at one of four hotspots (Berg et al., 1980; Engels and Preston, 1981a). The capacity of transposable elements in bacterial systems (see Kleckner, 1981, for a review) and mutable alleles in Drosophila (Lefevre and Green, 1972; Bingham, 1981; Lim, 1981) to promote the rearrangement of contiguous sequences is well established, and Berg et al. (1980) and Engels and Preston (1981a) proposed that the hotspots for rearrangement of the π_2 X chromosome were sites of residence of P factors.

We have examined the sites of residence of P

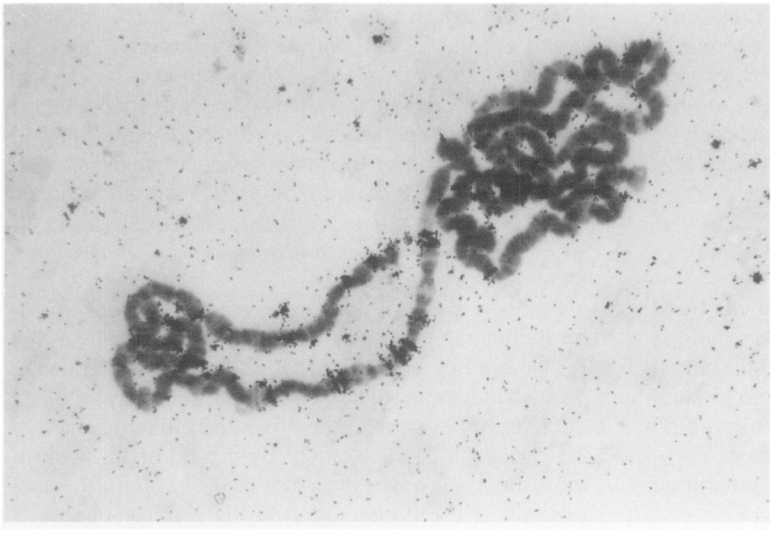

Figure 2. The Repeated Sequences Homologous to the P Element Are Dispersed

Shown is an in situ hybridization experiment. The chromosomes were isolated from the π_2 P strain. The sequence probe was the P-element-containing plasmid clone p6.1 (Experimental Procedures). Notice P element labeling sites on all major chromosome arms.

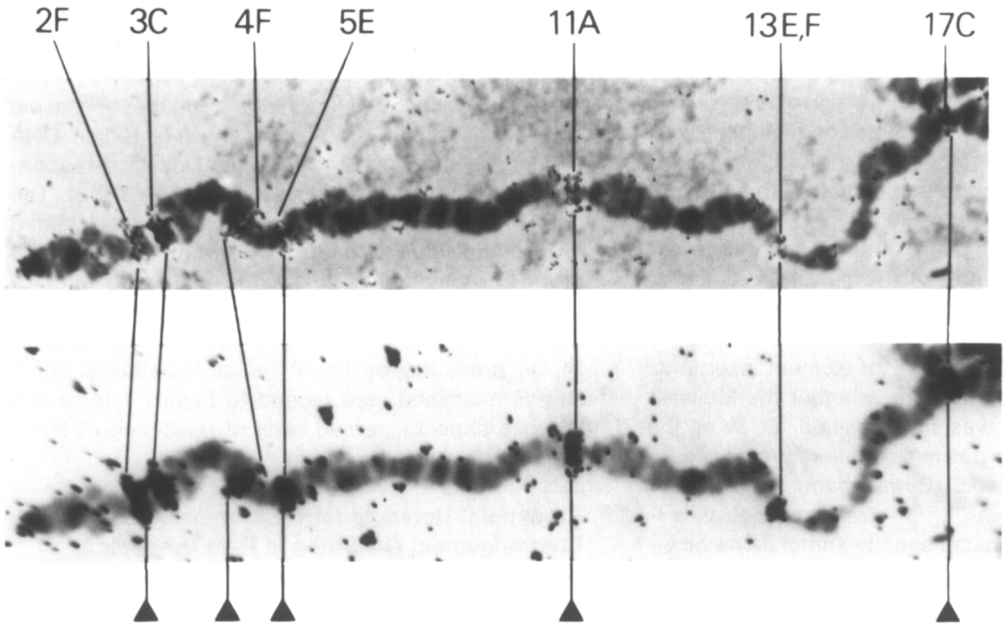

Figure 3. Chromosomal Rearrangement Breakpoint Hotspots Are Apparently Sites of Residence of P Elements

Shown is an in situ hybridization experiment. The chromosome shown is the X chromosome of the π_2(b) P strain, and the sequence probe is the P-element-containing plasmid clone p6.1 (Experimental Procedures). The top shows the chromosome photographed with phase-contrast optics to show the chromosomal banding pattern, and the bottom with bright-field optics to show the labeling sites. The cytological loci labeled by the probe are indicated by the number and letter combinations above the chromosome. The labeling in 3C is attributable to *white* region DNA sequences on the probe molecule, and all other sites of labeling are P elements. Triangles below the chromosome indicate the cytological loci of breakpoint hotspots for the rearrangement of this chromosome occurring in P–M dysgenic hybrids (Berg et al., 1980; Engels and Preston, 1981a; W. R. Engels and C. R. Preston, personal communication). A list of these breakpoint hotspots can be found in the text.

elements on t e X chromosome of the inbred subline of π_2, designated π_2(b), by in situ hybridization (Figure 3). The X chromosome of this strain carries the breakpoint hotspots observed in the parental π_2 X chromosome (at cytological loci 2F4-5, 5E3-7, 11A1 and 17C2-3), as well as an additional hotspot at cytological locus 4F9-10 (Engels and Preston, 1981a; W. R. Engels and C. R. Preston, personal communication). We observe six sites of P element labeling (in addition to the labeling in distal 3C attributable to *white* region sequences on the probe molecule) on the distal portion of the π_2(b) X chromosome, as well as one or more sites on the interval from proximal 18 through 20 (Figure 3). Five of these labeling sites (2F, 4F, 5E, distal 11A and 17C) are, within the resolution of cytological analysis, at the loci of the breakpoint hotspots of the π_2(b) X chromosome (Figure 3).

Copies of the P Element Apparently Transpose Frequently in P–M Dysgenic Hybrids

Genetic analysis of the acquisition of P activity by M X chromosomes occurring in P–M dysgenic hybrids (M. G. Kidwell, manuscript submitted) suggests that the P factor transposes at a substantial rate in such hybrids. This acquisition of P activity (as assayed by the capacity to induce sterility) is referred to as chromosome contamination (M. G. Kidwell, submitted manuscript).

We have carried out the following experiment to measure the rate of contamination of M X chromosomes in P–M dysgenic hybrid males. Using the mating scheme described in Experimental Procedures, we constructed P–M dysgenic hybrid males (F1 dysgenic males) whose X chromosomes were derived from a strain entirely devoid of detectable P element copies. Fifteen such F1 dysgenic males were crossed individually to attached-X females, and two male progeny from each such cross (F2 males) were analyzed as larvae by in situ hybridization to larval salivary gland chromosomes, with a P element sequence as probe (Figure 4 and Table 1). (In the special case of attached-X females, the male progeny of such females receive their X chromosome from their male parent.) Those squashes of F2 male larvae in which at least four individual X chromosomes were analyzable on the interval from the telomere through subdivision 18C were scored. Of 30 such preparations, 22 were analyzable by this criterion.

Figure 4 shows an example of an X chromosome of an F2 male larva, and in this case a single copy of the P element has been acquired by this X chromosome. Table 1 summarizes the results of the analysis of 22 such X chromosomes. We observed the acquisition of 18 copies of the P element by a total of 22 X chromosomes, corresponding to a rate of apparent transposition of the P element onto the X chromosome of

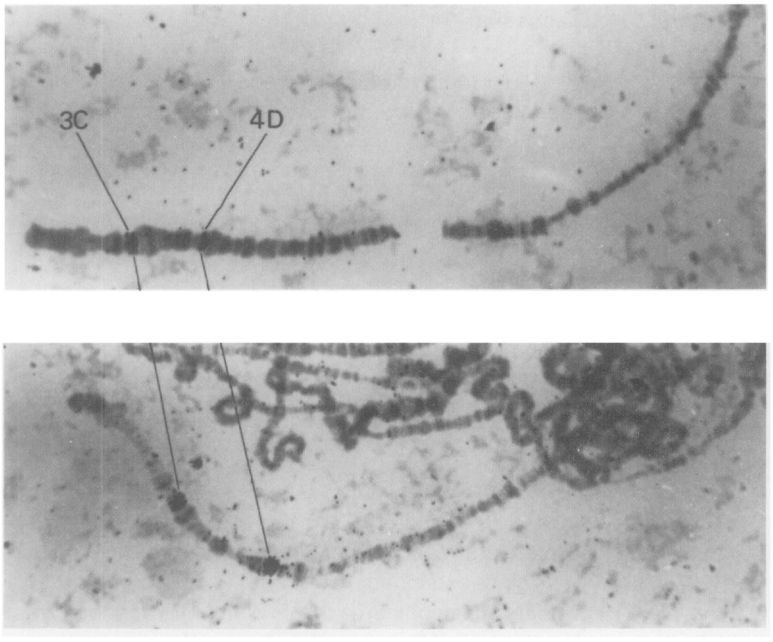

Figure 4. The Acquisition of Copies of the P Element by M Chromosomes in P–M Dysgenic Hybrids

Shown is an in situ hybridization experiment. The chromosomes are two X chromosomes from the same male individual. The sequence probe is the P-element-containing plasmid clone p6.1 (Experimental Procedures). The labeling site in 3C is attributable to *white* locus sequences on the probe molecule, and the site at 4D to P element sequences. See the text and Experimental Procedures for further details.

Table 1. Contamination of the Canton-S M X Chromosome in P–M Dysgenic Hybrid Males

Chromosome Serial No.	No. of P Element Labeling Sites	Loci of P Element Labeling Sites
1–9	0	2F–3A
10	1	2F–3A
11	1	4D–E
12	1	9C–D
13	1	18B
14	1	11A
15	1	5F–6A
16	1	18F
17	1	16D–F
18	2	14A; 9C–D
19	2	7C; 8A
20	2	11D–F; 19
21	2	16A–C; 18C
22	2	8A; 19

Results of analysis of 22 X chromosomes that were passaged through P–M dysgenic hybrid males (Experimental Procedures). The parental chromosome (before passage through a dysgenic hybrid) had no copies of the P element. The chromosomes have been numbered according to the number of P element copies acquired. A photograph of contaminated chromosome 11 is shown in Figure 4.

0.8 copies per fly generation. This measurement is in agreement with the rate of P factor transposition in dysgenic males estimated by genetic analysis (Engels and Preston, 1981a).

The question arises whether the acquisition of P element copies by M X chromosomes occurs only in the germline or occurs somatically as well. Fifteen F1 dysgenic males were analyzed in parallel with the 22 F2 dysgenic males described above. In no case was a copy of the P element detected on the salivary gland X chromosomes of such F1 dysgenic males. Thus the acquisition of P elements by M X chromosomes occurs more rarely, if at all, in the somatic cells ancestral to the larval salivary gland than in the germline dysgenic males. Collectively, the two sets of observations described immediately above are consistent with the hypothesis that the P element transposes in the germline tissues, but not in the somatic tissues, of dysgenic males. Various genetic experiments likewise suggest that the P factor is active exclusively in the germline (Engels, 1979b).

The Correlation between the Acquisition of P Elements by M X Chromosomes and the Capacity of Those Chromosomes to Induce Sterility in the M Cytotype

We wanted to determine whether M X chromosomes that have acquired P elements have also acquired the capacity to induce sterility in the M cytotype. The P factor hypothesis predicts that this should be the case. Genetic analysis suggests that the acquisition of P activity by M X chromosomes occurs substantially more frequently in P–M dysgenic hybrid females than in dysgenic males (M. G. Kidwell, manuscript submitted). For this reason, we tested for the correlation in question by examining M X chromosomes that had experienced a generation in a P–M dysgenic hybrid female.

The mating scheme used is described in Experimental Procedures. Essentially, M X chromosomes, having been contaminated by passage through a dysgenic female, are recovered individually as male progeny of such females. Individual contaminated X chromosomes are then tested by in situ hybridization for the acquisition of P elements and by crosses to the appropriate tester females for the capacity to induce sterility in the M cytotype (Experimental Procedures). The results of this experiment are diagrammed in Figure 5.

These results have two important features. First, the average number of P elements on the euchromatic portions of the 12 contaminated X chromosomes examined was 5.3. We observed a rate of acquisition of P elements by X chromosomes in dysgenic males of 0.8 per fly generation. These results are consistent with the hypothesis that the P element transposes more frequently in P–M dysgenic hybrid females than in the corresponding males. Second, we observe a correlation between the number of P elements acquired by an M X chromosome and the capacity of that chromosome to induce sterility in the M cytotype. Kendall's *tau* for these results is 0.733, which corresponds to a very strong correlation (Figure 5). The probability of a Kendall's *tau* of this absolute value or higher, assuming no correlation between the two parameters, is 0.00087. Although the correlation of these two parameters is very strong, it is not perfect (Figure 5). The imperfect nature of this correlation is susceptible to interpretation under a refined version of the P factor hypothesis (below).

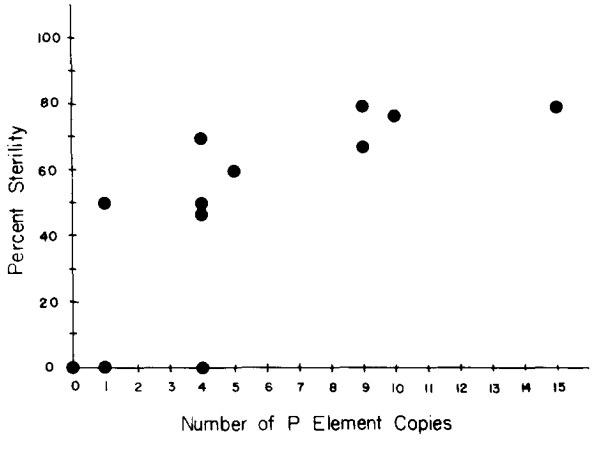

Figure 5. The Correlation between the Acquisition by X Chromosomes of P Element Copies and the Capacity to Induce Sterility in the M Cytotype

Plotted are two properties of formerly M X chromosomes that have been exposed to contamination by passage through a P–M dysgenic hybrid. The independent variable is the number of copies of the P element present on such chromosomes, as determined by in situ hybridization with a P-element-containing sequence probe (Experimental Procedures), and the dependent variable is the percentage of sterility induced by those chromosomes (Experimental Procedures).

Discussion

The P factor hypothesis makes several predictions amenable to experimental test. First, it predicts that mutant alleles arising in P–M dysgenic hybrids result from the insertion of members of a single transposon family. We have shown that this prediction is apparently fulfilled; four of five white mutant alleles arising in P–M dysgenic hybrids result from the insertion of elements having strongly homologous DNA sequence (Rubin et al., 1982). Second, it predicts that the transposon family whose members are responsible for inducing mutant alleles in P–M dysgenic hybrids will be a strain-specific family, present in P strains and missing from M strains. We have shown here that each of the four elements producing *white* mutant alleles arising in P–M dysgenic hybrids is homologous to a dispersed, repeated DNA sequence element family present in all P strains examined and missing from all M strains examined, with one exception. Third, it predicts that copies of the P element transpose frequently in P–M dysgenic hybrids. We have shown here that M X chromosomes acquire the P element at a rate of approximately 0.8 copies per fly generation in P–M dysgenic males and probably at an even higher rate in dysgenic females. Furthermore, formerly M X chromosomes that have acquired copies of the P element often acquire the capacity to induce sterility in the M cytotype. Moreover, we observe a correlation between the capacity of these chromosomes to induce sterility in the M cytotype and the number of P element copies acquired. Finally, it has been observed that P chromosomes, but not M chromosomes or chromosomal segments, undergo rearrangement at a very high rate in P–M dysgenic hybrids (Berg et al., 1980; Engels and Preston, 1981a; W. R. Engels and C. R. Preston, personal communication). The breakpoints for these P chromosome rearrangements have been proposed to be sites of residence of P factors (Berg et al., 1980; Engels and Preston, 1981a). We observe that each of five breakpoint hotspots for rearrangement of the π_2(b) P X chromosome is, within the resolution of cytological analysis, a site of residence of a P element.

A Refined Version of the P Factor Hypothesis

Collectively, the observations reported here and in the accompanying paper (Rubin et al., 1982) strongly support the P factor hypothesis and lead us to propose the following refined version of this hypothesis. A single transposon family is present in P strains and missing from M strains. (We will return to the possible implication of the exceptional M strain we have observed to have the P element family.) The presence of this strain-specific transposon family is is directly responsible for the P strain–M strain distinction. This family is highly heterogeneous, and its members are defined, at this point in the analysis, by virture of

strong DNA sequence homology to one another and to the P elements responsible for *white* mutant alleles arising in P–M dysgenic hybrids (Rubin et al., 1982). We propose that the heterogeneity we observe in the size and structure of P elements (Rubin et al., 1982) corresponds to heterogeneity in their genetic potential. In particular, we propose that some copies of the P element have one or more, while other copies lack one or more of the following properties: the capacity to express functions necessary for transposition (and for the possibly related process of stimulating chromosomal rearrangement), the capacity to induce sterility, the capacity to induce cytotype conversion from M to P and the capacity to act as substrate for transposition functions and, perhaps, for other functions as yet unrecognized. It is this refined version of the P factor hypothesis that leads us to use the term P element; we reserve the term P factor for P elements shown to have particular properties as assessed genetically (for example, the capacity to induce sterility in the M cytotype).

One attractive detailed version of this refined version of the P factor hypothesis proposes that the P element family originated as a single element copy having all functions now attributable to the aggregate of P elements, and that in some cases individual copies of the element have lost some or all of these capabilities by mutation.

This refined version of the P factor hypothesis is capable of rationalizing several additional observations. First, a class of strains, designated Q strains (Kidwell, 1979), are indistinguishable from P strains in all properties except one—the progeny of Q males crossed to M females, reared under conditions that would cause severe sterility among the progeny of P males and M females, are fully fertile (Kidwell, 1979; Engels and Preston, 1981b). Our proposal would account for the existence of Q strains by postulating that Q strains do not have copies of the P element that possesses the functional genetic elements which cause hybrid sterility, but do have P elements with all other properties attributable to the aggregate of P elements in P strains. Second, we have observed an exceptional M strain, the Muller-5 (Birmingham) strain, having the P element family as defined by hybridization assays. Our proposal accounts for this observation by supposing that all P elements present in this strain lack functional genetic elements necessary to produce the P cytotype and to cause sterility, mutation or male recombination in the M cytotype. Third, our proposal accounts for the imperfect nature of the correspondence between the number of P elements on freshly contaminated M chromosomes and the capacity of those chromosomes to induce sterility in the M cytotype (Figure 5). In particular, some P elements acquired by these chromosomes are presumed to lack functional genetic elements necessary to produce this sterility.

The Evolutionary Origin of the P Strain–M Strain Distinction

All strains that we have examined that have the P element family display 30–50 copies of the element dispersed among all the major chromosome arms, while all strains examined that lack the P element family apparently lack it utterly. Furthermore, strains descendant from gravid females caught in the wild more than 30 years ago are essentially always M, whereas strains descendant from such females caught in the wild during the last 10 years are usually P (Kidwell, 1979; Bregliano and Kidwell, 1982).

Two general hypotheses have been proposed to account for the origin of the P strain–M strain distinction (Kidwell, 1979, 1982; Engels, 1981): The "recent invasion" hypothesis proposes that the P element has invaded natural populations during the last 10–30 years. This invasion may have been initiated in any of several ways (for example, by a genetic transformation event or by infection by a virus-like entity). Subsequent spread of the element through the wild population is supposed to have occurred partly or entirely by vertical transmission involving the acquisition by formerly M chromosomes of P elements as a result of transposition. (Transposition of at least some transposons probably occurs by a process involving the generation of a new transposon copy rather than by excision of a transposon copy from one site, followed by its insertion at a second site; see Kleckner, 1981, for a review.) The recent invasion hypothesis thus supposes that most M strains lack the P element family as a result of its not having been present in the individuals originally trapped in the wild and ancestral to contemporary populations of M strains. The other hypothesis proposes no recent changes in natural populations but proposes that the P element family is frequently lost during laboratory culture of fly strains (for example, as a result of stochastic processes whose effects on small populations can be dramatic; Engels, 1981). These two hypotheses are not inherently mutually exclusive.

Several observations are relevant to evaluating these two hypotheses. First, Kidwell et al. (1981) have shown that laboratory populations artificially initiated with mixtures of P and M flies become predominantly P within a few generations, even when the initial mixture is predominantly M. Several observations suggested to these authors that the populations in question became predominantly P as a result, at least in part, of the conversion of M chromosomes to P chromosomes, presumably as a result of chromosome contamination (Kidwell et al., 1981). This observation suggests that the P element is invasive and supports the recent invasion hypothesis. Second, our measurements of the rate of acquisition of P elements by M X chromosomes in P–M dysgenic hybrids (Figures 4 and 5 and text above) further suggests that the P element is highly invasive. Third, P strains carried as

vial stocks (with small effective population sizes) have a measurable probability (~5% per stock per year) of undergoing conversion to M strains (W. R. Engels, personal communication). This observation can be interpreted to support the loss in laboratory culture hypothesis (W. R. Engels, personal communication); however, the observations described here lead us to point out an alternative interpretation. In particular, we suggest that the conversion of P strains to M strains under these conditions results not from the loss of the entire P element family, but rather from the loss (by segregation or mutation or both) of those members of the P element family not defective for genetic elements necessary to allow the strain to be P. Thus we speculate that M strains descendant from P strains will, in contrast to most M strains descendant from individuals trapped in the wild more than 30 years ago, possess the P element family as defined by hybridization assays. We further speculate that most or all of the rare M strains descendant from individuals trapped in the wild during the past several years (Kidwell, 1979) will, likewise, carry the P element family as defined by hybridization assays.

In summary, we suggest that these experimental observations favor the recent invasion hypothesis, though the issue is still unresolved.

Hybrid Dysgenesis and a Mechanistic Model for the Initiation of Speciation

It has been pointed out previously in general terms that various features of the hybrid dysgenesis syndrome suggest that this phenomenon may be involved in the establishment of the postmating reproductive isolation thought in some cases to represent the initiation of speciation (Kidwell, 1982). We point out a specific process whereby dysgenic phenomena may produce reproductive isolation of populations, and several considerations are important in this regard. In addition to the P–M system, a second dysgenic system, the I–R system, has been identified (see Bregliano et al., 1980, for a review). Genetic analysis strongly suggests that the I–R and P–M systems are distinct and independent (Kidwell, 1979). Our observation that IM strains (strains that are M in the P–M system and I in the I–R system) and RM strains (M in the P–M system and R in the I–R system) both lack homology to the P element strongly supports this supposition (Figure 1). While the P–M and I–R systems are distinct, they share several general properties. These include the property of partial sterility in the appropriate hybrids, and several features of the I–R system suggest that it is attributable to the properties of an I-strain-specific transposon family analogous to the P element (see Bregliano and Kidwell, 1982, for a review).

The observations reported here and the considerations discussed above lead us to propose that reproductive isolation of populations sometimes occurs by virtue of dysgenic phenomenona. A simple example is the recent invasion hypothesis; however, an equivalent model can be constructed from the assumption that element families are lost or both lost and acquired by wild populations. Populations are often invaded by (presumably parasitic) elements, of which the P element is an example. Furthermore, these elements often are capable of inducing partial sterility in the appropriate hybrids (either for unknown but general tactical reasons or as a common by-product of the processes engendered by these elements), as is observed in both the P–M and I–R cases. Moreover, the pool of distinct elements (source unknown) is sufficiently large and the probability of successful invasion sufficiently low that geographically isolated populations are not always invaded by the same element families. Finally, the aggregate effect of several dysgenic systems operating simultaneously is assumed to be effectively complete hybrid sterility.

A simple example of this process is as follows. A population is transiently divided into two geographically isolated subpopulations, X and Y. During this period of isolation, population X is invaded serially by element families A, B and C, and population Y is invaded by element families D, E and F. If the two populations again become sympatric, hybrids formed by the mating of males from population X with females from population Y are simultaneously dysgenic for the systems associated with the A, B and C families, and the offspring of the reciprocal mating are simultaneously dysgenic for the systems associated with the D, E and F families. By hypothesis, such multiply dysgenic individuals are effectively sterile.

The time scale of the P element invasion of the D. melanogaster population in the recent invasion hypothesis is of the order of 30 years. Thus processes of the sort we suggest are capable of producing reproductive isolation of populations geographically isolated for only very short periods of time. In particular, isolation processes of this sort seem likely to be far more rapid than genetic diversification of geographically isolated populations of the sort resulting in morphological or behavioral diversification adequate to produce reproductive isolation.

A General Strategy for the Cloning of Specific Gene Sequences

Bingham et al. (1981) proposed a general strategy applicable to the cloning of DNA sequences corresponding to genes in D. melanogaster identified by genetic analysis (including those for which no RNA product is known). This strategy employs mutant alleles at a desired locus, resulting from the insertion of a transposable element whose sequences have been previously cloned to retrieve the locus in question in cloned form. These authors suggested that if the P factor hypothesis were correct, the P element might represent the transposon of choice for this applica-

tion. The results described here and in Rubin et al. (1982) suggest that the use of the P element for this application is feasible. Moreover, the genetic studies of Simmons and Lim (1980) suggest that a substantial majority of complementation groups in Drosophila are susceptible to mutation by P element insertion.

Experimental Procedures

Drosphila Strains

A review of the properties of strains characterized with respect to their behavior in potentially dysgenic crosses can be found in Bregliano and Kidwell (1982), and descriptions of individual strains can be found in the text and in references cited in the text.

DNA Sequence Probes

Cloned segments from the *white* locus region have been described previously (Bingham et al., 1981; Levis et al., 1982). Cloned copies of the P element are described in Rubin et al. (1982). Plasmid clone p6.1 carries the 2.75 kb Sal I fragment carrying the P element insertion (1.2 kb) responsible for the w^{m6} *white* mutant allele and 1.55 kb of unique *white* region DNA sequences cloned into the Sal I site of pBR322 (Rubin et al., 1982). Nick translation of sequence probes was carried out as described by Bingham et al. (1981).

Purification of Drosophila DNA

Drosophila DNA was purified from adult flies as described by Bingham et al. (1981).

Southern Gel Analysis

Southern gel analysis was carried out according to the technique of Southern (1975) as modified by Botchan et al. (1976), with minor additions.

In Situ Hybridization

In situ hybridization was carried out as described by Bingham et al. (1981).

Mating Scheme for Contamination of M X Chromosomes in P-M Dysgenic Hybrid Males

Harwich (P) males were crossed to Canton-S (M) females at 22°C to produce dysgenic hybrid males (F1 dysgenic males) carrying the Canton-S X chromosome and heterozygous for Harwich and Canton-S autosomes. The genome of the Canton-S strain (including the X chromosome) has no detectable homology to the P element (Figure 1 and unpublished observations). To determine whether copies of the P element were acquired by the Canton-S X chromosome in the germline of F1 dysgenic males, such males were crossed to attached-X females at 22°C, and the male progeny (F2 males) were analyzed as larvae by in situ hybridization to the larval salivary gland chromosomes. (In the special case of attached-X females, the male progeny of such females receive their X chromosome from their male parent rather than from their female parent.) The sequence probe used in this experiment was the P-element-containing plasmid clone p6.1 (see DNA Sequence Probes).

Mating Scheme for Contamination of M X Chromosomes in Dysgenic Hybrid Females

The mating scheme used here is essentially identical to the mating scheme used to isolate X-linked mutant alleles arising in P-M dysgenic hybrid females, and a diagram of the mating scheme can be found in Rubin et al. (1982). The mating scheme is as follows. Harwich (P) males were crossed to H-40 (M) females. (The H-40 strain carries the Canton-S X chromosome and is heterozygous for the SM1 second chromosome balancer and the Ubx^{130} third chromosome balancer. Balancer chromosomes largely disrupt recombination such that, in an individual heterozygous for a second chromosome balancer, for example, the two second chromosomes of the diploid individual behave as Mendelian unit characters. (See Lindsley and Grell, 1968, for a detailed description of balancer chromosomes.) Male progeny (F1 males) from this mating and carrying the SM1 and Ubx^{130} chromosomes were selected. Such F1 males carry the Canton-S (M) X chromosome and carry the Harwich (P) autosomes heterozygous with the balancer second and third chromosomes and are dysgenic. F1 males were mated to H-40 females, and the female progeny carrying the SM1 and Ubx^{130} chromosomes were selected (F2 females). Such females are homozygous for the Canton-S (M) X chromosome and are heterozygous for the Harwich autosomes and the balancer second and third chromosomes. Such females are dysgenic, and previous genetic analysis suggests that the majority of the capacity to induce sterility in the M cytotype will be acquired in these F2 dysgenic females rather than in F1 dysgenic males (M. G. Kidwell, submitted manuscript). Virgin F2 dysgenic females were mated to M males, and the male progeny of this mating (F3 males) carrying the SM1 and Ubx^{130} balancer chromosomes were selected. Such males carry the Canton-S (M) X chromosome (now presumably contaminated) and lack either of the major Harwich (P) autosomes.

Individual contaminated M X chromosomes (from individual F3 males) were analyzed simultaneously for the acquisition of P element copies (by in situ hybridization) and for the capacity to induce sterility in the M cytotype as follows. Individual F3 males were mated serially to attached-X females at 22°C (to establish a fertile stock and to provide the F4 male progeny analyzed as larvae by in situ hybridization), to Canton-S females at 29°C (to produce female progeny who should show gonadal sterility if any of the chromosomes of the F3 male are contaminated with active P factors) and to attached-X females at 29°C (to produce female progeny who should show gonadal sterility only if the autosomes of F3 males have active P factors but not if all active P factor copies in F3 males are X-linked). These crosses allow the counting of P element copies on individual X chromosomes and also allow the contribution to sterility production by the autosomes and the X chromosome of F3 males to be analyzed separately. Under these circumstances the autosomes of F3 dygsenic males do not carry significant sterility-inducing capability; this capability is restricted to the X chromosome when present in the genome of an F3 male. Finally, individual F4 males (male progeny of cross of F3 male to attached-X female carried out at 22°C) were crossed serially to attached-X females and to Canton-S females, both at 29°C, to allow a second determination of the sterility-inducing capability acquired by each of the contaminated M X chromosomes examined.

The number of copies of the P element acquired by each contaminated chromosome analyzed was determined by examining the number of P element labeling sites on the larval salivary gland chromosomes of each of two F4 male progeny of individual F3 males. The probe used in this experiment was the P-element-containing plasmid clone p6.1. The sterility-inducing capability of individual contaminated X chromosomes is expressed as the percentage of rudimentary ovaries in a sample of 29 female progeny of F3 or F4 males mated to Canton-S females at 29°C. (See Kidwell, 1979, for a detailed description of the rudimentary ovaries associated with sterility in the P-M hybrid dysgenesis system.) The values used in constructing the plot shown in Figure 5 are the average values obtained from tests of the F3 male and one of his F4 male progeny.

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