

P Elements in Drosophila

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Introduction

The *Drosophila* genome has many families of transposable elements ([Flybase](#)), some of which have been studied in detail, and others are known only superficially ([Berg and Howe 1989](#)). Particular attention has been given to the P family (reviewed by [Engels 1989](#)), which has been the subject of intensive research for nearly two decades. There are two reasons for this special interest. First, the population biology and recent evolutionary history of P elements suggests a remarkable scenario of horizontal transfer from another species into *D. melanogaster* followed by rapid spread through the global population. The other reason is the wide array of technical applications that have made P elements an indispensable tool for manipulating the *Drosophila* genome.

1. P Element Structure

The structure of an autonomous P element is shown in Fig. 1 . The 2907-bp sequence (available from Genbank) features a perfect 31-bp terminal inverted repeat and an 11-bp subterminal inverted repeat (O'Hare and Rubin 1983). These repeats are needed in *cis* for efficient transposition, but they are not sufficient for it (Mullins, Rio and Rubin 1989). Internally, there are other repeat units of unknown function plus a *transposase* gene composed of four exons. This gene is required in *trans* for transposition, and part of the gene is also involved in regulation of P mobility (Rio 1990, Rio and Rubin 1988).

Nonautonomous P elements also exist. Some occur naturally through internal deletions of the autonomous elements, as shown in Fig. 1 . Such elements lack the *transposase* gene but retain the parts of the sequence required in *cis* for transposition. Mobilization of nonautonomous P elements occurs only if there is at least one autonomous P element present to supply *transposase*. Many artificial nonautonomous P elements have also been created in which the *transposase* gene has been replaced by another gene of interest, often functioning as a marker or reporter. Such elements are discussed further below.

2. Hybrid Dysgenesis

When P elements are mobilized they produce a syndrome of traits known collectively as hybrid dysgenesis (Kidwell, Kidwell and Sved 1977). These traits include temperature-dependent sterility, elevated rates of mutation, chromosome rearrangement, and recombination. The syndrome is usually seen only in the progeny of males with autonomous P elements and females that lack P elements. These two kinds of strains are called "P" and "M" because they contribute paternally and maternally respectively to hybrid dysgenesis. The reciprocal cross, P(female) x M(male), yields hybrids in which the dysgenic traits are much reduced, due to the maternal component of P element regulation by cytotype, as will be discussed below.

The dysgenic traits can be explained largely by genomic changes due to P element transposition and excision in developing germ cells. The sterility is due to loss of germ cells early in development (Engels and Preston 1979, Kidwell and Novy 1979, Niki 1986, Niki and Chigusa 1986, Wei, Oliver and Mahowald 1991). It is more pronounced in females, where there are fewer germ cells to spare than in males, and at temperatures above 25°C. The mutations come about through several mechanisms, but are primarily P insertions into genes and imprecise excision of P elements near genes (Rubin, Kidwell and Bingham 1982, Salz, Cline and Schedl 1987, Tsubota, Ashburner and Schedl 1985). Chromosome rearrangements usually result from breakage at the sites of two or more P element insertions, followed by rejoining of the chromosome segments in a different order (Engels and Preston 1981, Engels and Preston 1984, Roiha, Rubin and O'Hare 1988). P-induced recombination occurs preferentially in the genetic intervals containing mobile P elements (Sved et al. 1991, Sved, Eggleston and Engels 1990), and usually within 2 kb of the insertion site (Preston and Engels 1996, Preston, Sved and Engels 1996).

P element mobilization happens throughout development of the germline. Most mutations, rearrangements and recombination events occur prior to meiosis (Engels 1979, Hiraizumi 1979), but some meiotic events have also been detected (Daniels and Chovnick 1993). Premeiotic events tend to be recovered in clusters of two or more aberrant individuals among the progeny of a single dysgenic parent. The premeiotic timing of these events places a limitation on the genomic changes that can be recovered in the next generation, since the product must be cell-viable in the germline. Thus, mutations or rearrangements that do not yield viable germ cells in the parent will not

result in functional gametes, and therefore will not be recovered in the next generation, regardless of whether the hypothetical progeny bearing these changes would have been viable. In addition, premeiotic events result in frequency data that cannot be analyzed reliably by standard statistical methods based on Poisson or binomial distributions, and more robust alternatives must be employed (Engels 1979).

3. Population Biology

It is now widely believed that P elements have existed in the *D. melanogaster* genome for less than 100 years. According to this view, the elements were introduced through a rare horizontal transmission event in which one or more autonomous P element copies were acquired by *D. melanogaster* from another *Drosophila* species. The elements then spread strictly by heredity and transposition to become ubiquitous in natural populations within a few decades. This startling scenario was proposed by Kidwell (1979, 1983) and recently reviewed (see Engels 1992, Kidwell 1993) to explain the observation that the only true M strains were old laboratory stocks dating back to the early days of *Drosophila* genetics. Such strains would be reproductively isolated from natural populations, and thus escaped the P element invasion.

An alternative way to explain M strains was to postulate that the elements have existed in *D. melanogaster* over evolutionary time, but some aspect of laboratory culture conditions, such as small population size, acted to remove the P elements from the genome over several thousand generations (Engels 1981). The question was resolved when P elements from other *Drosophila* species were examined (Fig. 2). Some species closely related to *melanogaster* lacked P elements, but several much more distant relatives had them. In particular, the DNA sequence of a P element from *D. willistoni* was nearly identical to the *melanogaster* sequence, differing by only one base pair among 2907. Such conservation would be impossible over the 60 million years that the two species have diverged. It implies that P elements in the two species had a common ancestor in recent historical times.

D. melanogaster is now a cosmopolitan species, but it is thought to have evolved in western Africa (Lachaise et al. 1988). The species became established elsewhere only when human commercial shipping provided a means for long distance migration (Johnson 1913, Sturtevant 1921). Meanwhile, *D. willistoni* and related species evolved primarily in Central and South America, and are still endemic to these regions (Ashburner 1989). Therefore, they had no contact with *melanogaster* until the latter species arrived in the Americas. Johnson (1913), by examining antique insect collections, estimates that the first appearance of *D. melanogaster* in the New World occurred in the early 1800's, and they became widespread by the end of the century.

The horizontal transfer event could have occurred at any time since *melanogaster* and *willistoni* became sympatric, but the spread of P elements through *melanogaster* was presumably not yet complete by the 1930's when the last laboratory M populations were established (Kidwell 1983).

Currently, natural populations of *D. melanogaster* all appear to have P elements, including populations in such remote sites as the mountainous regions of central Asia (S. Nuzhdin and W. Engels, unpublished). However, the type and number of P elements show geographical differences (Anxolabéhère et al. 1984, Anxolabéhère, Kidwell and Périquet 1988, Anxolabéhère et al. 1985, Boussy et al. 1988, Kidwell, Frydryk and Novy 1983). For example, a North American population had 30-50 P elements in scattered chromosomal locations, with approximately two-thirds of them being nonautonomous

(O'Hare et al. 1992, O'Hare and Rubin 1983). Samples from other parts of the world, however, especially near the Mediterranean, show fewer P elements in the genome and a higher proportion of nonautonomous ones (Anxolabéhère et al. 1988, Anxolabéhère et al. 1985, Black et al. 1987). It is not known how such population differences are related to the invasion history of P elements.

The mechanism of the horizontal transfer between *D. willistoni* and *D. melanogaster* is also unknown. Speculation has focused on vector organisms, such as viruses and mites (Engels 1992, Houck et al. 1991). One parasitic mite species, *Proctolaelaps regalis*, has been studied as a possible DNA vector, and shows various features that make it compatible with this role (Houck et al. 1991). Once an autonomous P element had been introduced into *D. melanogaster* by whatever means, its ability to spread through the species was undoubtedly facilitated by a transposition mechanism in which DNA gap repair acts to increase the P element copy number, as discussed below.

P elements have a long evolutionary history in diptera prior to the invasion of *D. melanogaster* (Clark, Maddison and Kidwell 1994, Hagemann, Miller and Pinsker 1994, Lansman et al. 1987, Lansman et al. 1985). In the *D. willistoni* genome, for example, there are many "dead" P elements whose DNA sequences have accumulated numerous frameshifts and substitutions preventing them from either making transposase or serving as its substrate (Daniels et al. 1990). One species group was found to have a single genomic site where a portion of the P sequence was tandemly repeated (Miller et al. 1992, Paricio et al. 1991). This sequence lacked both P element termini and was incapable of encoding the P transposase, but it did encode a truncated transposase protein, which, as discussed below, acts as a repressor of P mobility. P-like elements have also been identified in several species of other genera and even outside the Drosophilidae family (Anxolabéhère, Nouaud and Périquet 1985, Anxolabéhère and Périquet 1987, Perkins and Howells 1992, Simonelig and Anxolabehere 1991). There is preliminary evidence that distant relatives of the P element are common among diptera (H. Robertson, personal communication).

Horizontal transfer and genomic invasion are probably not unusual in the world of transposable elements. The best example is that of the *mariner* element, which seems to have spread throughout the animal kingdom (Robertson 1993). However, horizontal transfer events involving *mariner*, though frequent on an evolutionary time scale, are typically separated by millions of years. It is notable, therefore, that P elements invaded *D. melanogaster* within a few decades after the opportunity arose, and spread throughout the species in less than 200 years.

What are the consequences to a species when a new transposable element invades its genome? Despite some arguments to the contrary (McDonald 1993, Syvanen 1984), most evidence suggests that the harmful mutations and chromosome rearrangements produced by transposition far outweigh any beneficial mutations that might also arise (Charlesworth and Langley 1989). In one series of experiments, P element invasion and rapid expansion in inbred laboratory M strains led to extinction of the lines within 20 generations (Preston and Engels 1989). The only exception was a case in which the population was expanded sufficiently to allow natural selection to eliminate deleterious insertions more efficiently. The ability of P elements to produce a negative regulator of their own mobility (discussed below) undoubtedly gave *D. melanogaster* a better chance of surviving its recent P element invasion by reducing the equilibrium copy number (Brookfield 1991, Charlesworth and Langley 1989).

4. Transposition

Several lines of evidence show that P elements transpose non replicatively and without an RNA intermediate (Engels et al. 1990, Kaufman and Rio 1992). The donor element is excised and reinserted into a recipient site creating a direct duplication of 8 bp at the site of insertion (O'Hare and Rubin 1983). The transposition reaction can be carried out in a cell-free system with partially purified transposase (Kaufman and Rio 1992), but host-encoded factors might also facilitate the reaction in vivo (Kaufman, Doll and Rio 1989).

4.1 Insertion site preference

P element insertions have been found at thousands of genomic positions, but not all sites are equally likely to be hit. The mechanism of insertion site selection is not known, but several generalizations can be made: (i) Euchromatic sites are hit more often than the heterochromatin (Berg and Spradling 1991, Engels 1989); (ii) Some euchromatic loci are much more susceptible to P mutagenesis than others. For example, the *singed* gene is hit at frequencies approaching 10^{-2} (Green 1977, Robertson et al. 1988, Simmons et al. 1984) whereas the *vestigial* gene has a rate of less than 10^{-6} (Williams and Bell 1988). Despite this variability, there is no evidence that any loci are immune from P element mutagenesis given a sufficiently large sample size; (iii) Within genes there is a preference for insertion in the non coding upstream sequences (Kelley et al. 1987); (iv) Target sites with close matches to the consensus octamer GGCCAGAC are more likely to receive P element insertions (O'Hare et al. 1992, O'Hare and Rubin 1983); (v) P elements tend to insert into or near other P elements, with a particular preference for base pairs 19-26 of the target P element (Eggleston 1990); (vi) Some P elements have been observed to jump preferentially to sites closely linked to the donor site (Golic 1994, Tower et al. 1993).

4.2 Transposase

The P element transposase is an 87 kiloDalton protein encoded by autonomous P elements (Fig. 1). It binds to subterminal regions at both ends of the element and represses transcription (Kaufman et al. 1989, Kaufman and Rio 1991). GTP is also bound by the transposase, and is required for transposition in vitro (Kaufman and Rio 1992).

4.3 Fate of the donor site

There is now considerable evidence that P element transposition leaves behind a double-strand DNA break. Sequences homologous to the flanking DNA are then copied in to repair the break (Engels et al. 1990, Gloor et al. 1991). The relative frequencies of transposition and excision suggest that approximately 85% of these repair events utilize a sister chromatid for the template (Engels et al. 1990). Thus the donor element is replaced by an identical P element copied in from the sister chromatid (Fig. 3). In such cases, the end result of transposition is a net gain of one P element copy. This net gain is probably responsible for the ability of P elements to increase their copy number in nature and in experimental populations (Engels 1992, Good et al. 1989, Kiyasu and Kidwell 1984, Meister and Grigliatti 1993, Preston and Engels 1989).

In the remaining 15% of repair events in which the sister chromatid is not used, the template can be either the homologous chromosome or an ectopic sequence, such as a transgene (Engels et al. 1990, Gloor et al. 1991). The homolog is used preferentially in such cases, especially if it contains a P element or fragment of a P element at the site corresponding to the break (Johnson-Schlitz and Engels 1993). The tendency to copy

from a P-bearing template might account for some of the preferential use of the sister chromatid, which necessarily contains a P element at the site. There is high sensitivity to mismatches between the sequence flanking the break and the template. Even 0.5% mismatches is sufficient to decrease the rate of repair three fold (Nassif and Engels 1993). Templates located on the same chromosome as the break are also used preferentially, even if the template lies on the opposite end of the chromosome (Engels, Preston and Johnson-Schlitz 1994).

Various types of imprecise excision can be attributed to aberrant repair. The most frequent such events are internal deletions resulting in structures similar to the nonautonomous P elements shown in Fig. 1. Breakpoints commonly occur at direct repeats of three or more base pairs, resulting in loss of one copy and the intervening sequence (Eggleston 1990, Engels 1989, O'Hare and Rubin 1983). When longer direct repeats are present, the frequency is greatly increased (Paques and Wegnez 1993). The most frequent kind of internal deletion leaves only 10-20 base pairs from each terminus, resulting in a non mobile "footprint" (Johnson-Schlitz and Engels 1993, Searles et al. 1982, Takasu-Ishikawa, Yoshihara and Hotta 1992). The term "internal deletion" might be a misnomer, since the events are probably due to a complete deletion of the P element followed by incomplete gap filling from the sister chromatid (Gloor et al. 1991). This interpretation is strengthened by the observation that when the P element resides on an extrachromosomal plasmid, and therefore lacks a sister chromatid for a template, the resulting footprints rarely contain more than four base pairs from each terminus (O'Brochta, Gomez and Handler 1991). The four or fewer bases that remain from each end could be explained if excision occurs by a staggered cut. Finally, imprecise P excisions that remove flanking DNA (Salz et al. 1987) can also be interpreted as aberrant repair events by assuming the gap left by a P element excision can be subsequently widened to varying degrees.

5. Regulation of P element mobility

P elements are not normally mobile in somatic cells, and their germline mobility does not occur within P strains. These two restrictions come about by different mechanisms.

5.1 Tissue specificity

Repression of P element transposition in somatic cells occurs on the level of RNA processing (Laski, Rio and Rubin 1986). The 2-3 intron (Fig. 1) is spliced only in the germ cells, resulting in the absence of transposase in somatic cells. Splicing of this intron is prevented in the somatic cells by a 97-kD protein that binds to a site in exon 2 located 12 to 31 bases from the 5' splice site (Chain et al. 1991, Siebel and Rio 1990, Tseng et al. 1991). When the 2-3 intron is removed artificially, the resulting transposase gene, designated Δ E2-3, produces functional transposase in both somatic and germline cells, and P elements are mobile in all tissues (Laski et al. 1986). This mobility results in pupal lethality if several mobile P elements are also present (Engels et al. 1987), especially in a DNA repair-deficient background (Banga, Velazquez and Boyd 1991).

5.2 Cytotype and repressors

The quiescent state of P elements within established P strains is best attributed to element-encoded repressor products. These repressors fall into at least two discrete categories, designated Type I and Type II.

5.2.1 Type I repressor

As noted earlier, P elements are repressed not only within P strains, but also in the hybrids of P(female) x M(male) crosses. They are not repressed in hybrids from the reciprocal cross (Kidwell et al. 1977), suggesting that repressor products produced in the P strain germline can be inherited maternally. Interestingly, this effect goes beyond simple maternal inheritance. Progeny from the cross MP(female) x P(male) show more P mobility than those from the cross PM(female) x P(male) (Engels 1979), where "MP" and "PM" represent the two reciprocal F1 hybrids with the female component shown first. Thus, the repressed state, called the P cytotype, is jointly determined by chromosomal and maternal components. One explanation proposed for this unusual inheritance was that the repressor-making P elements in the MP hybrids are more likely to be excised than those of the PM hybrids (Misra et al. 1993, Misra and Rio 1990). However, that explanation was ruled out by the finding that a single repressor-producing P element was sufficient for this mode of inheritance (Ronsseray, Lemaitre and Coen 1993). An alternative model (Lemaitre, Ronsseray and Coen 1993) involving differential splicing of the same intron that is involved in tissue-specific regulation, provides an adequate explanation for available data, and will be discussed further below.

Various lines of evidence indicated that the 66 kD truncated transposase protein that is made when the 2-3 intron is unspliced functions as a repressor of P mobility (Gloor et al. 1993, Handler, Gomez and O'Brochta 1993, Misra et al. 1993, Misra and Rio 1990, Rio, Laski and Rubin 1986). Several other truncated transposase molecules with breakpoints in slightly different places can also function in this way (Gloor et al. 1993, Robertson and Engels 1989). Fine structure deletion mapping revealed that the minimal 3' boundary for this kind of repressor was between nucleotides 1950 and 1956 of the P element sequence (Gloor et al. 1993). These repressors are designated Type I to distinguish them from a class of much smaller truncated transposase proteins called Type II, discussed below.

The use of a reporter gene fused to the P element's transposase promoter showed that this repression acted on the level of transcription (Lemaitre and Coen 1991, Lemaitre et al. 1993). This effect also provided an explanation for several observations that P repressors affected expression of genes neighboring P element insertion sites (Coen 1990, Engels 1979, Gloor et al. 1993, Robertson and Engels 1989, Williams, Pappu and Bell 1988). Finally, the transcriptional regulation revealed by the reporter gene showed a maternal effect in the germline but not in somatic cells (Lemaitre et al. 1993).

These observations led Lemaitre *et al.* (1993) to propose a model in which the 97-kD protein previously shown to prevent splicing of the 2-3 intron in somatic cells was also present germinally, but in much reduced quantities. Thus, in the P cytotype, transcription of the transposase message is relatively low, and the available germline splice blocker is sufficient to ensure that only 66-kD product is made. This 66-kD product serves as a transcriptional repressor in the germline to perpetuate the P cytotype through the female lineage as long as there are sufficiently many repressor-producing P elements on the chromosomes. In the M cytotype, the level of expression is increased sufficiently to overwhelm the splice-blocking agent in the germline, and functional 87-kD transposase is made. This model requires a nonlinear relationship between transcript level and the ratio of 66 to 87 kD products in order to explain the maintenance of M cytotype.

5.2.2 Type II repressor elements

The number of distinct nonautonomous P elements in nature is so large that few have

been observed in more than one population (O'Hare et al. 1992, O'Hare and Rubin 1983). The first and most conspicuous exception to this rule is the KP element, which is very common worldwide (Black et al. 1987). It was therefore suggested that KP elements might function as P element repressors, and thus be favored by natural selection (Black et al. 1987, Jackson, Black and Dover 1988). This possibility was verified when KP elements were isolated genetically and tested for repressor (Rasmusson, Raymond and Simmons 1993). KP elements did not fit the paradigm of Type I elements for two reasons: First, they had a deletion for nucleotides 808-2560 (Fig. 1), and thus lacked the minimal sequence previously shown to be required for Type I repressors (Gloor et al. 1993). Second, the repression in KP lines showed none of the maternal inheritance associated with cytotype (Rasmusson et al. 1993, Raymond et al. 1991). This lack of maternal inheritance is expected under the splice-blocking model of cytotype described above because the 2-3 intron is not present in KP elements.

It soon became clear that KP was not the only other element of this kind. The D50 element had similar repressor properties but slightly different deletion endpoints (Rasmusson et al. 1993). To date, five such elements, designated Type II repressor-makers, have been identified, at least four of which are geographically widespread (C. Preston, G. Gloor and W. Engels, unpublished). All have large deletions whose endpoints are similar to (within 300 bp) the KP deletion endpoints. A base substitution at either nucleotide position 32 or 33 is also present in most Type II repressors.

It is not known whether Type II regulation works by the same mechanism as Type I, but they do share several features in common. Type II regulation probably also acts on the level of transcription, since the elements can reduce the expression of a reporter gene fused to the P promoter (Lemaitre et al. 1993), and they display the secondary effects on expression of genes closely linked to P insertions (C. Preston and W. Engels, unpublished). For both types, there is a pronounced position effect such that the ability of the element to function as a repressor is highly sensitive to its genomic insertion site (Gloor et al. 1993, Higuete, Anxolabéhère and Nouaud 1992, Misra et al. 1993, Robertson and Engels 1989, Ronsseray, Lehmann and Anxolabéhère 1991).

6. P elements as molecular biological tools

Drosophila has long been a favorite organism for genetic and developmental research, but it was largely through the use of P elements that the powerful tools of molecular biology were fully employed. P elements are used for identifying genes of interest, cloning them, and for placing them back into the genome. There are several key features of P element biology that make them especially well suited for these roles. The existence of M strains allows experimenters to create stocks containing only selected P elements. Transposase can easily be added or removed genetically. The high mobility of P elements and their retention of this mobility despite drastic modifications to their internal sequences are also essential features. Most recently, the double-strand DNA breaks created by P element excision have been used to effect gene replacement and to study the repair process.

6.1 Mutagenesis

A basic problem in genetics has long been how to obtain molecular information for a gene known only by the phenotype of its mutations. The reverse problem, obtaining mutations in a gene known only by its DNA sequence is also becoming increasingly common. Oddly enough, both problems often have the same solution for *Drosophila* geneticists: obtain a P element insertion into the gene. In the former case, the P insertion

allows cloning by transposon tagging (Bingham, Kidwell and Rubin 1982, Searles et al. 1982), and, in the latter, P element insertion mutations can be selected by PCR-based methods without knowledge of the phenotype (see below). For these reasons, the search for P element insertion mutations engages much of the effort of *Drosophila* workers regardless of their particular biological focus (Kidwell 1986).

The most efficient approach to P element mutagenesis utilizes an immobile copy of the transposase gene combined with one or more mobile, but nonautonomous, P elements to serve as "ammunition". The P{ry+ 2-3}(99B) element is an example of an immobile transposase source that has been widely used for P mutagenesis (Robertson et al. 1988). Its transposase gene lacks the 2-3 intron, thus precluding the production of any 66 kD protein, which, as discussed above, functions as a repressor. This element cannot transpose due to a deletion of one of its termini (H. Robertson, personal communication). A cross with the transposase source coming from one parent and the ammunition elements from the other yields progeny in whose germ cells mutagenesis occurs. The transposase source is then eliminated by segregation in the next generation to stabilize any mutations obtained.

There are two general strategies in the selection of ammunition elements. One is to use a chromosome carrying as many highly mobile elements as possible, such as the Birm2 chromosome (Engels et al. 1987, Robertson et al. 1988) which has 17 small nonautonomous P elements from nature. This approach maximizes the likelihood of obtaining the desired mutation, but it is often laborious to isolate the mutation from the rest of the P elements in the genome. Alternatively, one can use a smaller number of artificially constructed P elements (Cooley, Kelley and Spradling 1988). This method usually requires a larger screen, but any mutation obtained is easier to isolate, especially if the ammunition element(s) carries a bacterial origin of replication and selectable marker to permit cloning by plasmid rescue.

PCR can be employed to screen for P insertions if no phenotypic screen is available but the target gene has been cloned. DNA is extracted from a pool of potential mutants and amplified with one primer in the P element sequence and the other in the targeted DNA. A P element insertion close to the targeted site is required to bring sites for these two primers together, and yield amplification (Ballinger and Benzer 1989, Kaiser and Goodwin 1990). The target size of this approach is necessarily small because PCR cannot amplify more than a few kb. An alternative approach that permits a much larger target size is provided by inverse PCR (Sentry and Kaiser 1994). Here the two primers are both within the P element sequence, but directed away from each other. Amplification can occur when the newly inserted P element, along with some of the flanking DNA is circularized following digestion with a restriction enzyme and ligation (Ochman, Gerber and Hartl 1988). DNA amplified in this way from a pool of potential mutants can then be probed with target DNA of arbitrary length, and insertion mutations can be identified.

Once a P insertion has been obtained in or near the gene of interest, additional genetic variability can be generated readily by the reintroduction of transposase. Internal deletions or **flanking deletions** can be selected (Salz et al. 1987, Tsubota and Schedl 1986). In some cases, transposase can catalyze an event in which one P element in the genome is substituted for another P element elsewhere in the genome by an unknown mechanism (Staveley et al. 1994). This process can be useful for putting a reporter gene into a specific site.

6.2 P element mediated transformation

The most important use of P elements is undoubtedly that of making transgenic flies (Rubin and Spradling 1982, Spradling and Rubin 1982). The gene of interest is placed between P element ends, usually within a plasmid, and injected into pre-blastoderm embryos in the presence of transposase. This P element, with the gene as cargo, then transposes from the plasmid to a random chromosomal site. Technical aspects of the method have been described elsewhere (Ashburner 1989, Spradling 1986). In a typical experiment, 10-20% of the fertile injected flies produce transformant progeny.

The P element may also carry a second gene used to identify transformants. The frequency of transformation is usually sufficiently great that a visible marker, such as an eye color gene (Pirrotta 1988, Rubin and Spradling 1982) is more efficient than a selectable marker, such as neomycin resistance. The size of the inserted sequence can exceed 40 kb (Haenlin et al. 1985), but such large vectors come at a cost of decreased transformation frequency. In some cases, the sequence carried by the P element can influence the transformation rate (Spradling 1986) or the insertion site specificity (Kassis et al. 1992).

There are several options for providing transposase to the injected DNA. One way is to bind purified transposase protein to the element prior to injection (Kaufman and Rio 1992). However, the difficulty in obtaining transposase in sufficient quantities usually makes this method impractical. Alternatively, one can coinject a transposase-making "helper" plasmid, preferably one that is unable to integrate into the chromosomes itself (Karess and Rubin 1984). A third approach is to inject directly into embryos that have an endogenous transposase source, such as the P{ry+ 2-3}(99B) element mentioned previously (Robertson et al. 1988). The transposase-bearing chromosome can be marked with a dominant mutation, and stable transformants lacking the transposase gene are then selected among the progeny. This procedure is probably more efficient than coinjection, since it does not require the embryonic nuclei to take up two independent plasmids.

6.3 Enhancer trapping

P element mobility also provides a way to sample the genome for loci whose expression matches a particular pattern (O'Kane and Gehring 1987). A lacZ reporter gene is fused to a weak promoter and mobilized within a P element to produce a collection of *Drosophila* lines, each with a single insertion of the "enhancer trap" element at a random site. The expression pattern of lacZ in each line tends to reflect the expression of nearby genes. Thus, one can identify genes that are active in specific tissues and developmental periods. The power of this technique increases rapidly with time, as large collections of enhancer trap lines become available (eg., Hartenstein and Jan 1992), thus eliminating the need for each worker to produce a new collection.

6.4 P vectors for transgene expression

Several P element vectors are available to facilitate expression of a given gene in a particular tissue through fusion of the gene to a specific promoter. For example, one set of vectors includes a promoter for strong expression in the developing egg and early embryo (Serano et al. 1994). A particularly versatile system employs a two-element combination to allow a given gene to be expressed in any of a wide variety of patterns (Brand and Perrimon 1993). One element in this combination is similar to the enhancer trap construct discussed above except that the reporter gene is the yeast transcriptional activator, GAL4. The second element carries the gene of interest driven by a promoter containing GAL4 binding sites. The gene is then activated only in the cells where GAL4

is expressed. Thus, the expression of the gene of interest depends on the insertion site of the P{GAL4} element.

6.5 P vectors for site-specific recombination

Another yeast system that has proven useful in *Drosophila* is the FLP site-specific recombinase, and its target site, FRT (Golic and Lindquist 1989). This system is particularly useful for generating mosaics. One P element carries the FLP recombinase gene driven by a heat shock promoter, and a second element has a gene with two FRT sites embedded. When heat shock is applied to such flies, FLP-mediated recombination causes somatic loss of the gene carrying FRT sites. More recently, FLP has been used to generate somatic mosaics with sectors homozygous for an entire chromosome arm (Xu and Rubin 1993). A homozygous P element near the base of a chromosome arm and bearing an FRT site undergoes mitotic recombination when FLP is expressed. The result is a somatic sector that is homozygous for all genes distal to the FRT-bearing P element. Such sectors can be identified by absence of a cell-autonomous marker present on one of the homologs. This method allows identification and analysis of genes that are lethal when homozygous in the whole organism.

6.6 Gene replacement

With P element mediated transformation, as described above, the researcher has no control over where in the genome the construct goes. In many instances, however, what is needed is to replace genes *in situ*. For example, some genes are too large to manipulate *in vitro* and return to the genome by transformation. Others are too sensitive to position effects. In addition, some genes have no null alleles to provide a suitable background to test transgenes.

To achieve gene replacement, *Drosophila* geneticists can make use of P-induced double strand breaks (Gloor et al. 1991). The method requires construction of an altered version of the gene which will be used as the template for gap repair. This construct must contain the sequences flanking the P insertion site. As discussed earlier the gaps produced by P element excision are usually repaired by copying in sequences from the sister strand (Fig. 3). However, in approximately 15% of the cases, the homolog or an ectopic sequence can provide the template (Engels et al. 1990). This method has been tested most extensively in the *white* gene, where hundreds of gene replacement events have been analyzed, but it has also been used successfully at *forked* (Lankenau, Corces and Engels 1996) and at least two other loci (Papoulas, McCall and Bender 1994). The frequencies of gene replacement measured with *white* were dependent on the genomic position of the template, averaging 1% for autosomal sites (Gloor et al. 1991) and 6% for X-linked sites (Engels et al. 1994). Extrachromosomal templates have also been used (G. Gloor, personal communication, Banga and Boyd 1992, Papoulas et al. 1994). Insertions and deletions could be copied into the gap just as efficiently as single base pair changes (Johnson-Schlitz and Engels 1993, Nassif et al. 1994).

The primary limitation of this technique is its requirement for a P insertion close the site being modified. A site within 8 bp of the P insertion in *white* was replaced in close to 100% of the gene replacement events, but one 2 kb away was replaced less than 10% of the time. To a good approximation, the replacement frequency of a site n bp away from the P insertion was $0.99855n$, expressed as a proportion of the gap repair events (Gloor et al. 1991). Therefore, the P mutagenesis techniques discussed above, especially the PCR-based screens (Ballinger and Benzer 1989, Kaiser and Goodwin 1990, Sentry and Kaiser

1994), are particularly valuable as preliminary steps toward gene replacement.

6.7 Making flanking deletions

Recent work has shown that an efficient way to obtain deletions of the sequences flanking a P element is by selection for male recombination events (Preston et al. 1996). The mechanism is thought to be the "half-element insertion" (HEI) process postulated from work on end-deleted P elements (Gray, Tanaka and Sved 1996, Svoboda, Robson and Sved 1995).

If you are using Netscape Navigator 2 or later you can see how the HEI model works to produce recombination by [viewing the animated diagram](#). In addition, HEI can explain P-induced inversions on nonrecombinant chromosomes. There is also an [animated inversion diagram](#) available for viewing.

The following points are useful in planning the use of this technique:

- **The Cross:** Set up males with a transposase source (such as 2-3), the P element near your target gene, and a pair of heterozygous flanking markers. Then screen for male recombinants among the progeny. It doesn't matter how far apart the markers are -- it can be the length of the entire chromosome -- because the great majority of recombination will occur at the P element site.)
- **Frequency:** Expect about 0.5 - 1% of the progeny to be recombinants.
- **Temperature:** Grow flies at 25°C, especially the generation in whose germline the recombination is to occur. This appears to be the optimum temperature for 2-3 activity.
- **Direction:** Select the direction of the deletion by picking which reciprocal recombinant to use. Namely, in the heterozygous male shown at right, look for rightward deletions among the Ab recombinants, and leftward deletions in the aB recombinants.
- **Deletion Fraction:** Expect that about 1/3 of the recombinants will have a deletion. (The rest are evenly split between duplications and "simple" recombinants.)
- **Retention of P:** Most deletions will retain a functional P element. In some cases this retention of a functional P means that the deletion-bearing chromosome can be used for additional rounds of deletion generation to create more variability. The deletion begins at the P element boundary.
- **Stabilization:** Because of this functional P element, it is important to select recombinants that lack transposase in order to avoid subsequent changes. (Since the overall recombination rate will be around 1% of all progeny, it is feasible to throw out the half of them that receive 2-3.)
- **Lengths:** The [deletion sizes](#) range from a few base pairs to at least several hundred kb in length. Some knocked out three or more genetic loci. We could [see the larger ones cytologically](#).
- **Using a Second Round:** Larger deletions can be made by a second round of recombination starting with a deleted chromosome created by the above method. The deletion used must retain a complete P element to initiate a second recombination. It is possible to extend the original deletion to enhance the frequency of obtaining a knockout of a distant locus. This occurs in approximately 1/3 of the new recombinants. The [sizes of second-round deletions](#) obtained in our experiment are shown.
- **Complications:**
 - If you have an X chromosomal target locus, you need to do the experiment in females in order to have crossing over. This requires closely linked flanking markers.
 - If your locus is on chromosome 3 and you use P{w+ 2-3}(99B) as the transposase

source, it is preferable that the mobile P element comes from one parent and 2-3 comes from the other to avoid premature mobilization. If the mobile P element happens to lie to the left of the target gene, this means the desired recombinant will have 2-3, which violates suggestion 7 above. One workaround is to use a different transposase source on another chromosome. Alternatively, you can just put up with the extra generation of transposase.

- **Alternative Approach:** Similar HEI events can occur between sister chromatids, leading to duplications and deletions among the nonrecombinant chromosomes. If you have a good way to screen for these without relying on flanking marker crossing over, you can pick up the desired deletion on nonrecombinant chromosomes that retain the mobile P element.

7. Conclusions

P elements are relative newcomers in the *Drosophila melanogaster* genome, probably arriving through a horizontal transfer event less than 200 years ago. Their invasion of the genome was almost certainly harmful to the species, lowering the average fitness throughout natural populations. However, P elements have undoubtedly enhanced the fitness of *Drosophila* geneticists, forming the basis for a variety of techniques that have become essential to most current research with this species.

Acknowledgment

Christine R. Preston provided many comments and suggestions in preparation of this review.

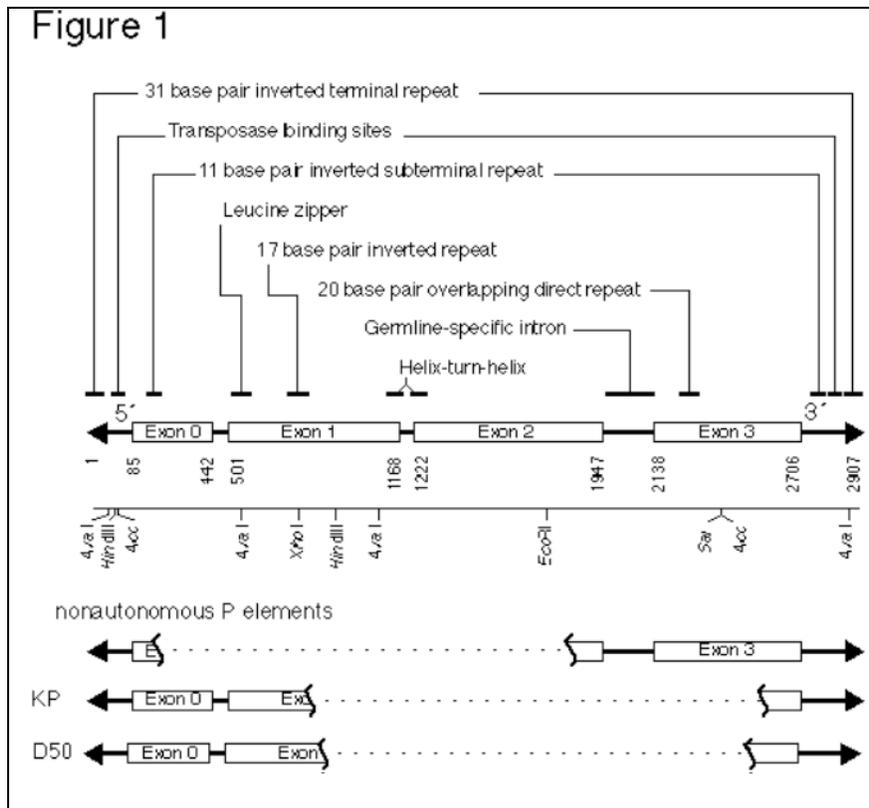


Fig. 1. P element anatomy (modified from Lindsley and Zimm 1992) : An autonomous P element is shown with some of its sequence features and a restriction map. Three examples of nonautonomous P elements, including the Type II repressor-making elements, KP and D50. For the complete P element sequence, see the [Genbank entry](#) provided by [O'Hare and Rubin \(1983\)](#).

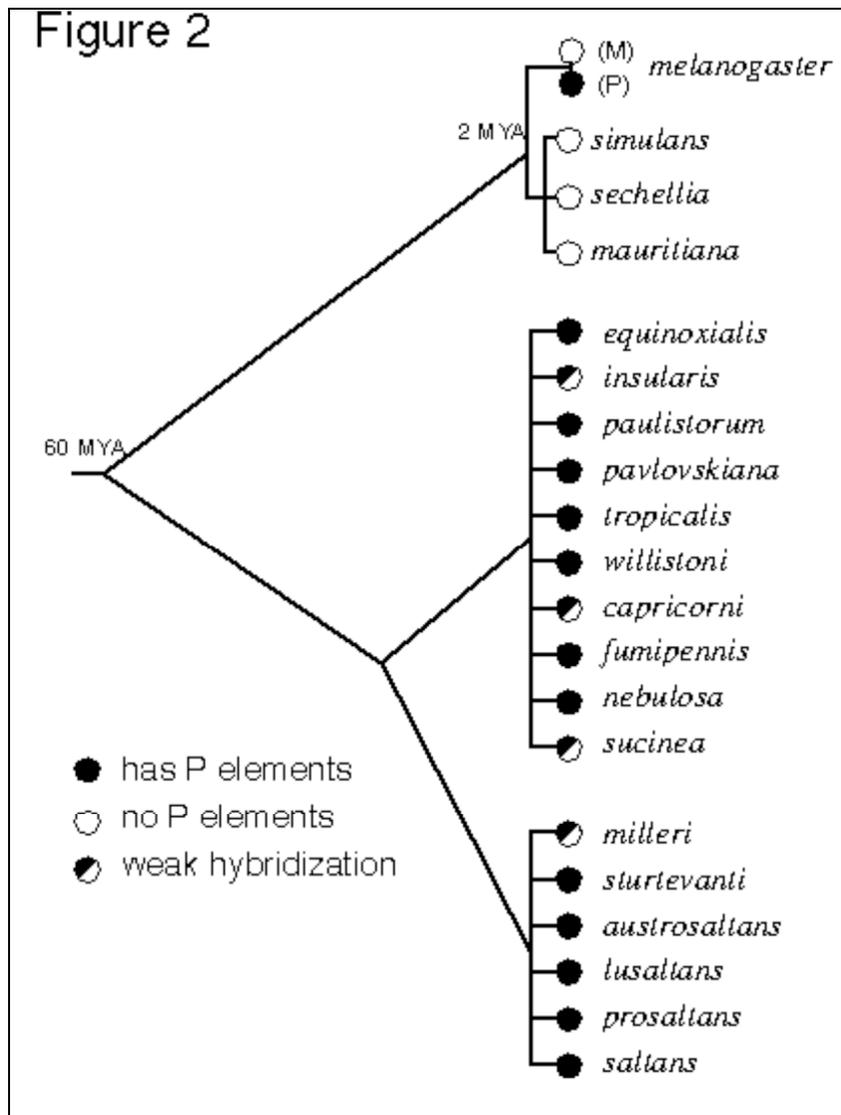


Fig. 2. Species distribution of P elements (modified from [Engels 1992](#)) : Southern blotting was used to determine the presence or absence of P element sequences in various *Drosophila* species to illustrate the evidence for recent invasion ([Daniels et al. 1990](#) and references cited therein) . Estimates of divergence time, given in millions of years ago, are averages derived from [Lachaise et al. \(1988\)](#) and Spicer (1988).

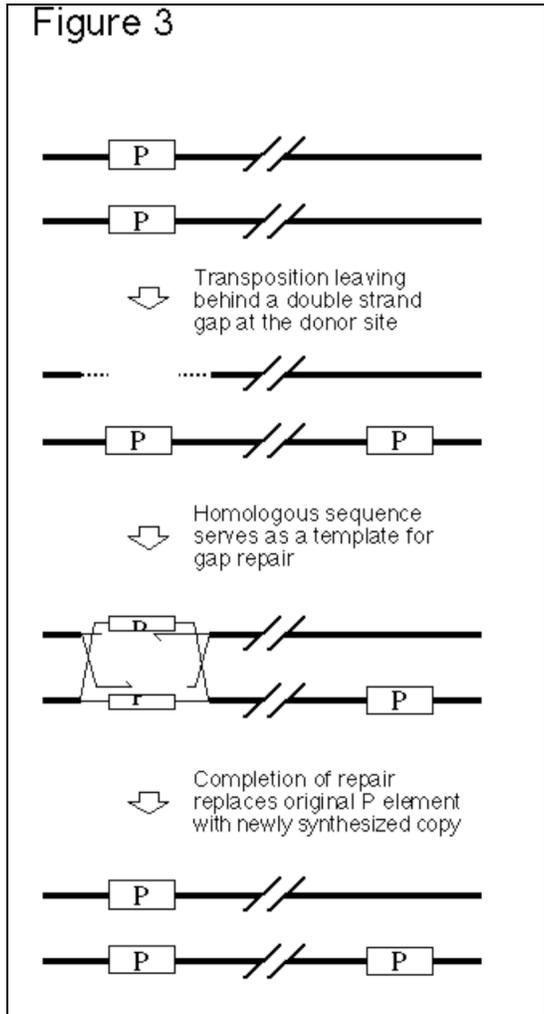


Fig. 3. P element transposition and gap repair (modified from [Engels 1992](#)) : The P element on one sister strand jumps to a new site leaving behind a double strand gap. Repair utilizes the other sister strand resulting in a net gain of one P element copy ([Engels et al. 1990](#)).

P Elements Bibliography

- **Anxolabéhère, D., H. Kai, D. Nouaud, G. Périquet and S. Ronsseray.** 1984. The geographical distribution of P-M hybrid dysgenesis in *Drosophila melanogaster*. *Génét. Sél. Evol.* **16**:15-26.
- **Anxolabéhère, D., M. G. Kidwell and G. Périquet.** 1988. Molecular characteristics of diverse populations are consistent with a recent invasion of *Drosophila melanogaster* by mobile P elements. *Mol. Biol. and Evol.* **5(3)**:252-269.
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- **Anxolabéhère, D. and G. Périquet.** 1987. P-homologous sequences in diptera are not restricted to the Drosophilidae family. *Genetica Iberica* **39**:211-222.
- **Ashburner, M.** 1989. *Drosophila*, A laboratory handbook. Cold Spring Harbor Press, Cold Spring Harbor, New York
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- **Ballinger, D. G. and S. Benzer.** 1989. Targeted gene mutations in *Drosophila*. *Proc. Nat. Acad. Sci. USA* **86**:9402-9406.
- **Banga, S. S. and J. B. Boyd.** 1992. Oligonucleotide-directed site-specific mutagenesis of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci.* **89**:1735-1739.
- **Banga, S. S., A. Velazquez and J. B. Boyd.** 1991. P transposition in *Drosophila* provides a new tool for analyzing postreplication repair and double-strand break repair. *Mutat. Res.* **255**:79-88.

A genetic screen has been developed in *Drosophila* for identifying host-repair genes responsible for processing DNA lesions formed during mobilization of P transposable elements. Application of that approach to repair deficient mutants has revealed that the *mei-41* and *mus302* genes are necessary for recovery of P-bearing chromosomes undergoing transposition. Both of these genes are required for normal postreplication repair. Mutants deficient in excision repair, on the other hand, have no detected effect on the repair of transposition-induced lesions. These observations suggest that P element-induced lesions are repaired by a postreplication pathway of DNA repair. The data further support recent studies implicating double-strand DNA breaks as intermediates in P transposition, because the *mei-41* gene has been genetically and cytologically associated with the repair of interrupted chromosomes. Analysis of this system has also revealed a striking stimulation of site-specific gene conversion and recombination by P transposition. This result strongly suggests that postreplication repair in this model eukaryote operates through a conversion/recombination mechanism. Our results also support a recently developed model for a conversion-like mechanism of P transposition (Engels et al., 1990). Involvement of the *mei-41* and *mus302* genes in the repair of P element-induced double-strand breaks and postreplication repair points to a commonality in the mechanisms of these processes.

- **Berg, C. A. and A. C. Spradling.** 1991. Studies on the rate and site-specificity of P element transposition. *Genetics.* **127**:515-524.

A single genetically marked P element can be efficiently mobilized to insertionally mutagenize the *Drosophila* genome. We have investigated how the structure of the starting element and its location along the X chromosome influenced the rate and location of mutations recovered. The structure of two P[*rosy+*] elements strongly affected mobilization by the autonomous "Jumpstarter-1" element. Their average transposition rates differed more than 12-fold, while their initial chromosomal location had a smaller

effect. The lethal and sterile mutations induced by mobilizing a P[rosy+] element from position 1F were compared with those identified previously using a P[neoR] element at position 9C. With one possible exception, insertion hotspots for one element were frequently also targets of the other transposon. These experiments suggested that the genomic location of a P element does not usually influence its target sites on nonhomologous chromosomes. During the course of these experiments, Y-linked insertions expressing rosy+ were recovered, suggesting that marked P elements can sometimes insert and function at heterochromatic sites.

- **Berg, D. E. and M. M. Howe.** 1989. Mobile DNA. American Society of Microbiology, Washington, D.C.
- **Bingham, P. M., M. G. Kidwell and G. M. Rubin.** 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.
- **Black, D. M., M. S. Jackson, M. G. Kidwell and G. A. Dover.** 1987. KP elements repress P-induced hybrid dysgenesis in *D. melanogaster*. *EMBO J* **6**:4125-4135.
- **Boussy, I. A., M. J. Healy, J. G. Oakeshott and M. G. Kidwell.** 1988. Molecular analysis of the P-M gonadal dysgenesis cline in eastern Australian *Drosophila melanogaster*. *Genetics* **119**:889-902.
- **Brand, A. H. and N. Perrimon.** 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development*. **118**:401-415.

We have designed a system for targeted gene expression that allows the selective activation of any cloned gene in a wide variety of tissue- and cell-specific patterns. The gene encoding the yeast transcriptional activator GAL4 is inserted randomly into the *Drosophila* genome to drive GAL4 expression from one of a diverse array of genomic enhancers. It is then possible to introduce a gene containing GAL4 binding sites within its promoter, to activate it in those cells where GAL4 is expressed, and to observe the effect of this directed misexpression on development. We have used GAL4-directed transcription to expand the domain of embryonic expression of the homeobox protein even-skipped. We show that even-skipped represses wingless and transforms cells that would normally secrete naked cuticle into denticle secreting cells. The GAL4 system can thus be used to study regulatory interactions during embryonic development. In adults, targeted expression can be used to generate dominant phenotypes for use in genetic screens. We have directed expression of an activated form of the Dras2 protein, resulting in dominant eye and wing defects that can be used in screens to identify other members of the Dras2 signal transduction pathway.

- **Brookfield, J.** 1991. Models of repression of transposition in P-M hybrid dysgenesis by P cytotype and by zygotically encoded repressor proteins. *Genetics* **128**:471-486.
- **Chain, A. C., S. Zollman, J. C. Tseng and F. A. Laski.** 1991. Identification of a cis-acting sequence required for germ line-specific splicing of the P element ORF2-ORF3 intron. *Mol. Cell. Biol.* **11**:1538-1546.
- **Charlesworth, B. and C. H. Langley.** 1989. The population genetics of *Drosophila* transposable elements. *Annu. Rev. Genet.* **23**:251-287.
- **Clark, J. B., W. P. Maddison and M. G. Kidwell.** 1994. Phylogenetic Analysis Supports Horizontal Transfer of P Transposable Elements. *Mol. Biol. and Evol.* **11**:40-50.

Nucleotide sequence comparisons were used to investigate the evolution of P transposable elements and the possibility that horizontal transfer has played a role in their occurrence in natural populations of *Drosophila* and other Diptera. The phylogeny of P elements was examined using published sequences from eight dipteran taxa and a new, partial sequence from *Scaptomyza elmoi*. The results from a number of different analyses are highly consistent and reveal a P-element phylogeny that contradicts the phylogeny of the species. At least three instances of horizontal transfer are necessary to explain this

incongruence, but other explanations cannot be ruled out at this time.

- **Coen, D.** 1990. P element regulatory Products enhance *zeste1* repression of a P[*whiteduplicated*] transgene in *Drosophila melanogaster*. *Genetics* **126**:949-960.
- **Cooley, L., R. Kelley and A. Spradling.** 1988. Insertional mutagenesis of the *Drosophila* genome with single P elements. *Science* **239**:1121-1128.
- **Daniels, S. B. and A. Chovnick.** 1993. P element transposition in *Drosophila melanogaster*: an analysis of sister-chromatid pairs and the formation of intragenic secondary insertions during meiosis. *Genetics*. **133**:623-636.

The gap-repair model proposes that P elements move via a conservative, "cut-and-paste" mechanism followed by double-strand gap repair, using either the sister chromatid or homolog as the repair template. We have tested this model by examining meiotic perturbations of an X-linked *ry+* transposon during the meiotic cycle of males, employing the *mei-S332* mutation, which induces high frequency equational nondisjunction. This system permits the capture of both sister-X chromatids in a single patroclinous daughter. In the presence of P-transposase, transpositions within the immediate proximity of the original site are quite frequent. These are readily detectable among the patroclinous daughters, thereby allowing the combined analysis of the transposed element, the donor site and the putative sister-strand template. Molecular analysis of 22 meiotic transposition events provide results that support the gap-repair model of P element transposition. Prior to this investigation, it was not known whether transposition events were exclusively or predominantly premeiotic. The results of our genetic analysis revealed that P elements mobilize at relatively high frequencies during meiosis. We estimated that approximately 4% of the dysgenic male gametes have transposon perturbations of meiotic origin; the proportion of gametes containing lesions of premeiotic origin was estimated at 32%.

- **Daniels, S. B., K. R. Peterson, L. D. Strausbaugh, M. G. Kidwell and A. Chovnick.** 1990. Evidence for horizontal transmission of the P transposable element between *Drosophila* species. *Genetics* **124**:339-355.

Several studies have suggested that P elements have rapidly spread through natural populations of *Drosophila melanogaster* within the last four decades. This observation, together with the observation that P elements are absent in the other species of the *melanogaster* subgroup, has led to the suggestion that P elements may have entered the *D. melanogaster* genome by horizontal transmission from some more distantly related species. In an effort to identify the potential donor in the horizontal transfer event, we have undertaken an extensive survey of the genus *Drosophila* using Southern blot analysis. The results showed that P-homologous sequences are essentially confined to the subgenus *Sophophora*. The strongest P hybridization occurs in species from the closely related *willistoni* group. A wild-derived strain of *D. willistoni* was subsequently selected for a more comprehensive molecular examination. As part of the analysis, a complete P element was cloned and sequenced from this line. Its nucleotide sequence was found to be identical to the *D. melanogaster* canonical P, with the exception of a single base substitution at position 32. When the cloned element was injected into *D. melanogaster* embryos, it was able to both promote transposition of a coinjected marked transposon and induce singed-weak mutability, thus demonstrating its ability to function as an autonomous element. The results of this study suggest that *D. willistoni* may have served as the donor species in the horizontal transfer of P elements to *D. melanogaster*.

- **Eggleston, W. B.** Thesis: 1990. P element transposition and excision in *Drosophila*: Interactions between elements. *University of Wisconsin*, *Genetics* 203.

The objective of this thesis work was to examine P element excision transposition in *Drosophila melanogaster*. This was done by characterizing dysgenesis-induced mutations of P element insertion alleles at two loci with a combination of genetic and molecular biology tools.

One result to arise from this work was that sites with integrated *P* elements can frequently be the sites of additional *P* element insertions. It was concluded integrated *P* elements not only influenced the sites of nearby insertions, but their presence actually increased the rate of insertions into the region. While it was not clear what the basis for these effects were, it was strongly suggested *P* element-induced chromatin changes may have been involved. The data were also interpreted as suggesting *P* elements made a significant number of short range transpositions. However, much more work is needed on this question before a strong conclusion can be made.

A second result was the quality and quantity of excisions were quite different for alleles with a single *P* element and alleles with two closely inserted *P* elements. When only a single element was present, excisions were invariably imprecise, leaving some portion of the element at the site. The total excision rate for alleles with a single *P* element were in the range of one to a few percent, while precise excision rates were less than a fraction of a percent. Conversely, excisions from alleles with two *P* elements inserted into the same target site were almost entirely precise, and occurred at rates as high as 50%.

However, all alleles with closely inserted elements did not behave similarly. While alleles with two *P* elements inserted into the same target site were highly mutable, undergoing precise excisions at high rates, this was only true of some of the alleles where the two elements were not inserted into the same target site. The basis for the difference was concluded to be related to the sequences flanking the two elements and potentially the distance between the elements. The results suggested similar target site duplications flanking two nearby elements were essential for high mutation and precise excision rates.

The data on excisions of solo elements was uninformative in terms of distinguishing amongst the various models of *P* element excision. However, in combination with preliminary data from an experiment designed to measure precise excision rates, it was postulated excisions of single elements resulted from a precise excision of the element followed by annealing or template-dependent repair and then annealing of the broken ends.

The data on "precise excisions" from alleles with two elements separated by more than 8 bp confirmed predictions made based on one of the models by [Roiha, Rubin and O'Hare \(1988\)](#) to explain the behavior of the prototype double insertion allele, *snw*. The loss of sequences between separated *P* elements led to the conclusion that "precise excisions" from closely inserted elements resulted from transposase-dependent pairing of the terminal repeats and potentially flanking DNA, followed by an exchange event or replication fork slippage in the region. Consequently, it was concluded the mechanism responsible for losses of single *P* elements was distinct for the losses of one of two closely inserted *P* elements.

The results and materials that arose from the work in this thesis have explored previously unrecognized facets of *P* element biology. These data could form the basis for additional experiments to study how *P* elements interact with each other and with host genomes in order to better understand their evolutionary impact on the organisms in which they are found.

- **Engels, W. R.** 1979. The estimation of mutation rates when premeiotic events are involved. *Environmental Mutagenesis* **1**:37-43.

When mutation or recombination events occur premeiotically, the distribution of exceptional individuals among the offspring will be "clustered" as opposed to binomial. Even though the exact nature of the clustering is usually unknown, unbiased methods for measuring mutation rate and determining the precision of these measurements are given to replaced a biased method now frequently used. When clustering is pronounced, the unweighted average mutation rate is found to be a more efficient estimator than the usual average weighted by family size. Methods of statistical inference and optimal experimental design in the absence of specific knowledge of the mechanism of clustering are also discussed.

The recommended estimators for the variance are:

where pw is the frequency estimate (total number of mutants / total scored); ni is the number of individuals scored in group i ; mi is the number of mutants in group i ; n and m are the sums of ni and mi over all groups scored.

Equation (5) is best in situations where the mutation rate (pw) is close to 0 or 1, and equation (A6) is more accurate when pw is closer to 1/2.

- **Engels, W. R.** 1979. Extrachromosomal control of mutability in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci. USA* **76**:4011-4015.

Hybrid dysgenesis is a syndrome of germ-line aberrations including, e.g., sterility and mutation, found in certain interstrain hybrids of *Drosophila melanogaster*. Previous studies of sterility have shown that elements responsible for dysgenesis may reside on all major chromosomes, but that their dysgenesis-causing ability is controlled by an unknown extrachromosomal factor. Dysgenic hybrids also give rise to unstable visible mutations thought to be DNA insertions at certain sensitive loci. One such unstable allele at the singed bristle locus, designated *snw*, was found to mutate at extraordinary rates exceeding 50%. This instability was shown to be under the same extrachromosomal control as hybrid dysgenesis itself. That is, the mutability of *snw* was reversibly suppressed when placed in the background cytotype known to prevent sterility and other characteristics of hybrid dysgenesis. These results suggest that *snw* may represent an insertion at the singed locus of a hypothetical gene responsible for hybrid dysgenesis.

- **Engels, W. R.** 1979. Germline aberrations associated with a case of hybrid dysgenesis in *Drosophila melanogaster* males. *Genet. Res. Camb.* **33**:137-146.
- **Engels, W. R.** 1979. Hybrid dysgenesis in *Drosophila melanogaster*: Rules of inheritance of female sterility. *Genet. Res. Camb.* **33**:219-236.
- **Engels, W. R.** 1981. Hybrid dysgenesis in *Drosophila* and the stochastic loss hypothesis. *Cold Spring Harbor Symp. Quant. Biol.* **45**:561-565.
- **Engels, W. R.** 1989. P elements in *Drosophila*. pp. 437-484 in *Mobile DNA*, edited by D. Berg and M. Howe. American Society of Microbiology, Washington, D.C.

No Abstract Available: This is a longish review -- about 37,000 words -- on all aspects of the biology of P elements covering reports up to 1987.

- **Engels, W. R.** 1992. The origin of P elements in *Drosophila melanogaster*. *BioEssays* **14**:681-686.

The P family of transposable genetic elements is thought to be a recent addition to the *Drosophila melanogaster* genome. New evidence suggests that the elements came from another *Drosophila* species, possibly carried by parasitic mites. The transposition mechanism of P elements involves DNA gap repair which may have facilitated their rapid spread through *D. melanogaster* worldwide. These results provide new insight into the process of a transposon's invasion into a new species and the potential risk of extinction such an invasion might entail.

- **Engels, W. R., W. K. Benz, C. R. Preston, P. L. Graham, R. W. Phillis and H. M. Robertson.** 1987. Somatic effects of P element activity in *Drosophila melanogaster*: Pupal lethality. *Genetics* **117**:745-757.

Nonautonomous P elements normally excise and transpose only when a source of transposase is supplied, and only in the germline. The germline specificity depends on one of the introns of the transposase gene which is not spliced in somatic cells. To study the effects of somatic P activity, a modified P element (2-3) lacking this intron was used as a source of transposase. Nonautonomous P elements from a strain called Birmingham, when mobilized in somatic cells by 2-3, were found to cause lethality, although neither component was lethal by itself. The three major Birmingham chromosomes acted

approximately independently in producing the lethal effect. This lethality showed a strong dependence on temperature. Although temperature sensitivity was limited to larval stages, the actual deaths occurred at the pupal stage. Survivors, which could be recovered by decreasing the temperature or by reducing the proportion of the Birmingham genome present, often showed multiple developmental anomalies and reduced longevity reminiscent of the effects of cell death from radiation damage. Although the genetic damage occurred in dividing imaginal disc cells, the phenotypic manifestations--death and abnormalities--are not observed until later. The survivors also showed gonadal dysgenic (GD) sterility, a well-known characteristic of P-M hybrid dysgenesis. To explain these findings, we suggest that pupal lethality and GD sterility are both caused by massive chromosome breakage in larval cells, resulting from excision and transposition of genomic P elements acting as substrate for the transposase.

- **Engels, W. R., D. M. Johnson-Schlitz, W. B. Eggleston and J. Sved.** 1990. High-frequency P element loss in *Drosophila* is homolog-dependent. *Cell* **62**:515-525.
P transposable elements in *Drosophila melanogaster* can undergo precise loss at a rate exceeding 13% per generation. The process is similar to gene conversion in its requirement for a homolog that is wild type at the insertion site, and in its reduced frequency when pairing between the homologs is inhibited. However, it differs from classical gene conversion by its high frequency, its requirement for P transposase, its unidirectionality, and its occurrence in somatic and pre-meiotic cells. The results suggest a model of P element transposition in which jumps occur by a "cut-and-paste" mechanism, but are followed by double-strand gap repair to restore the P element at the donor site. The results also suggest a technique for site-directed mutagenesis in *Drosophila*.
- **Engels, W. R. and C. R. Preston.** 1979. Hybrid dysgenesis in *Drosophila melanogaster*: The biology of male and female sterility. *Genetics* **92**:161-175.
High levels of female and male sterility were observed among the hybrids from one of the two reciprocal crosses between a wild strain of *D. melanogaster* known as pi2 and laboratory strains. The sterility, which is part of a common syndrome called hybrid dysgenesis, was found to be associated with the rudimentary condition of one or both of the ovaries or testes. All other tissues, including those of the reproductive system were normal, as were longevity and mating behavior. The morphological details of the sterility closely mimic the agametic condition occurring when germ cells are destroyed by irradiation or by the maternal-effect mutation, grandchildless. We suggest that sterility in hybrid dysgenesis is also caused by failure in the early development of germ cells. There is a thermo-sensitive period beginning at approximately the time of initiation of mitosis among primordial germ cells a few hours before the egg hatches and ending during the early larval stages. Our results suggest that hybrid dysgenesis, which also includes male recombination, mutation and other traits, may be limited to the germ line, and that each of the primordial germ cells develops, or fails to develop, independently of the others. This hypothesis is consistent with the observed frequencies of unilateral and bilateral sterility, with the shape of the thermosensitivity curves and with the fact that males are less often sterile than females. The features of this intraspecific hybrid sterility are found to resemble those seen in some interspecific *Drosophila* hybrids, especially those from the cross *D. melanogaster* X *D. simulans*.
- **Engels, W. R. and C. R. Preston.** 1981. Identifying P factors in *Drosophila* by means of chromosome breakage hotspots. *Cell* **26**:421-428.
A syndrome of germline abnormalities in *Drosophila melanogaster* called hybrid dysgenesis is thought to be caused by transposable genetic elements known as P factors. Several lines of evidence presented here show that the chromosomal positions of at least some P factors can be identified as points of frequent chromosome breakage (hotspots).

Starting with a strain (pi 2) in which four hotspots had been identified on the X chromosome, we found individual hotspots vanished when their part of the chromosome was replaced by the homologous part from a strain known to lack P factors. All hotspots in the non-substituted parts of the chromosome remained functional, indicating that they can act autonomously. We also observed a new breakage site coinciding with the appearance of an unstable mutation at the singed bristle locus (*snw*). This mutation was dysgenesis-induced, and previous genetic evidence suggested that it was caused by the insertion of a P factor at that locus. We also present preliminary evidence for rapid scrambling of the positions of hotspots under certain conditions, and we describe a new procedure for efficiently determining the positions of hotspots on a given chromosome.

- **Engels, W. R. and C. R. Preston.** 1984. Formation of chromosome rearrangements by P factors in *Drosophila*. *Genetics*. **107**:657-678.

We studied a collection of 746 chromosome rearrangements all induced by the activity of members of the P family of transposable elements in *Drosophila melanogaster*. The chromosomes ranged from simple inversions to complex rearrangements. The distribution of complex rearrangement classes was of the kind expected if each rearrangement came about from a single multibreak event followed by random rejoining of chromosome segments, as opposed to a series of two-break events. Most breakpoints occurred at or very near (within a few hundred nucleotide pairs) the sites of preexisting P elements, but these elements were often lost during the rearrangement event. There were also a few cases of apparent gain of P elements. In cases in which both breakpoints of an inversion retained P elements, that inversion was capable of reverting at high frequencies to the original sequence or something close to it. This reversion occurred with sufficient precision to restore the function of a gene, *heldup-b*, which had been mutated by the breakpoint. However, some of the reversions had acquired irregularities at the former breakpoints that were detectable either by standard cytology or by molecular methods. The revertants themselves retained the ability to undergo further rearrangements depending on the presence of P elements. We interpret these results to rule out the simplest hypotheses of rearrangement formation that involve cointegrate structures or homologous recombination. The data provide a general picture of the rearrangement process and its possible relationship to transposition.

- **Engels, W. R., C. R. Preston and D. M. Johnson-Schlitz.** 1994. Long-range *cis* preference in DNA homology search extending over the length of a *Drosophila* chromosome. *Science* **263**:1623-1625.

P element-induced chromosome breakage on the X chromosome of *Drosophila melanogaster* was repaired six times more frequently when a homologous template was located anywhere on the X rather than on an autosome. Cis-trans comparisons confirmed that recombinational repair was more frequent when the interacting sequences were physically connected. These results suggest that the search for homology between the broken ends and a matching template sequence occurs preferentially in the cis configuration. This cis advantage operates over more than 15 megabases of DNA.

- **Gloor, G. B., N. A. Nassif, D. M. Johnson-Schlitz, C. R. Preston and W. R. Engels.** 1991. Targeted gene replacement in *Drosophila* via P element-induced gap repair. *Science* **253**:1110-1117.

Transposable elements of the P family in *Drosophila* are thought to transpose by a cut-and-paste process that leaves a double-strand gap. The repair of such gaps resulted in the transfer of up to several kilobase pairs of information from a homologous template sequence to the site of P element excision by a process similar to gene conversion. The template was an *in vitro*-modified sequence which was tested at a variety of genomic positions. Characterization of 123 conversion tracts provided a detailed description of

their length and distribution. Most events were continuous conversion tracts that overlapped the P insertion site without concomitant conversion of the template. The average conversion tract was 1379 base pairs, and the distribution of tract lengths fit a simple model of gap enlargement. The conversion events occurred at sufficiently high frequencies to form the basis of an efficient means of directed gene replacement.

- **Gloor, G. B., C. R. Preston, D. M. Johnson-Schlitz, N. A. Nassif, R. W. Phillis, W. K. Benz, H. M. Robertson and W. R. Engels.** 1993. Type I repressors of P element mobility. *Genetics* 135:81-95.

We describe here a family of P elements that we refer to as Type I repressors. These elements are identified by their repressor functions and their lack of any deletion within the first two-thirds of the canonical P sequence. Elements belonging to this repressor class were isolated from P strains and were made *in vitro*. We found that Type I repressor elements could strongly repress both a cytotype-dependent allele and P element mobility in somatic and germline tissues. These effects were very dependent on genomic position. Moreover, we observed that an element's ability to repress in one assay positively correlated with its ability to repress in either of the other two assays. The Type I family of repressor elements includes both autonomous P elements and those lacking exon 3 of the P element. Fine structure deletion mapping showed that the minimal 3' boundary of a functional Type I element lies between nucleotide position 1950 and 1956. None of 12 elements examined with more extreme deletions extending into exon 2 made repressor. We conclude that the Type I repressors form a structurally distinct group that does not include more extensively deleted repressors such as the KP element described previously.

- **Golic, K. and S. Lindquist.** 1989. The FLP recombinase of yeast catalyzes site-specific recombination in the *Drosophila* genome. *Cell* 59:499-509.
- **Golic, K. G.** 1994. Local transposition of P elements in *Drosophila melanogaster* and recombination between duplicated elements using a site-specific recombinase. *Genetics* 137:551-63.

The transposase source 2-3(99B) was used to mobilize a P element located at sites on chromosomes X, 2 and 3. The transposition event most frequently recovered was a chromosome with two copies of the P element at or near the original site of insertion. These were easily recognized because the P element carried a hypomorphic white gene with a dosage dependent phenotype; flies with two copies of the gene have darker eyes than flies with one copy. The P element also carried direct repeats of the recombination target (FRT) for the FLP site-specific recombinase. The synthesis of FLP in these flies caused excision of the FRT-flanked white gene. Because the two white copies excised independently, patches of eye tissue with different levels of pigmentation were produced. Thus, the presence of two copies of the FRT-flanked white gene could be verified. When the P elements lay in the same orientation, FLP-mediated recombination between the FRTs on separated elements produced deficiencies and duplications of the flanked region. When P elements were inverted, the predominant consequence of FLP-catalyzed recombination between the inverted elements was the formation of dicentric chromosomes and acentric fragments as a result of unequal sister chromatid exchange.

- **Good, A. G., G. A. Meister, H. W. Brock, T. A. Grigliatti and D. A. Hickey.** 1989. Rapid spread of transposable P elements in experimental populations of *Drosophila melanogaster*. *Genetics* 122:387-396.
- **Gray, Y., M. Tanaka and J. A. Sved.** 1996. P element-induced recombination in *Drosophila melanogaster*: hybrid element insertion. *Genetics* (in press)

It has previously been shown that the combination of two deleted P elements in trans, one containing the left functional end and the second element the right functional end, can lead to high levels of male recombination. This finding strongly suggests that P element ends from different chromosomes can become associated, followed by 'pseudo-excision',.

We show that the structure formed from this excision event can be resolved in two ways: (1) the excised P element ends continue to function as a single unit (Hybrid Element) and insert at a nearby site in the chromosome or into the element itself (Hybrid Element Insertion - HEI), (2) free ends which may or may not contain P element ends repair and re-join (Hybrid Excision and Repair - HER). Both types of resolution can lead to recombination, and this paper concentrates on the HEI class. One type of HEI event predicts the exact reverse complementary duplication of an 8bp target site, and we have confirmed the existence of such a structure in a number of recombinant chromosomes. There is also a high tendency for insertion events to occur within a few bases of the original 8bp target site, including six apparent cases of insertion into the exact site. The results suggest that where insertion occurs close to an element, it is preferentially near the functional end of the element rather than the non-functional end.

(Accompanying papers by [Preston & Engels](#), [Preston](#), [Sved & Engels](#))

- **Green, M. M.** 1977. Genetic instability in *Drosophila melanogaster*: De novo induction of putative insertion mutations. *Proc. Nat. Acad. Sci. USA* **74**:3490-3493.
- **Haenlin, M., H. Steller, V. Pirrotta and E. Mohier.** 1985. A 43 kilobase cosmid P transposon rescues the *fs(1)K10* morphogenetic locus and three adjacent *Drosophila* developmental mutants. *Cell* **40**:827-837.
- **Hagemann, S., W. J. Miller and W. Pinsker.** 1994. Two distinct P element subfamilies in the genome of *Drosophila bifasciata*. *Mol Gen Genet* **244**:168-75.

The genome of *Drosophila bifasciata* harbours two distinct subfamilies of P-homologous sequences, designated M-type and O-type elements based on similarities to P element sequences from other species. Both subfamilies have some general features in common: they are of similar length (M-type: 2935 bp, O-type: 2986 bp), are flanked by direct repeats of 8 bp (the presumptive target sequence), contain terminal inverted repeats, and have a coding region consisting of four exons. The splice sites are at homologous positions and the exons have the coding capacity for proteins of 753 amino acids (M-type) and 757 amino acids (O-type). It seems likely that both types of element represent functional transposons. The nucleotide divergence of the two P element subfamilies is high (31%). The main structural difference is observed in the terminal inverted repeats. Whereas the termini of M-type elements consists of 31 bp inverted repeats, the inverted repeats of the O-type elements are interrupted by non-complementary stretches of DNA, 12 bp at the 5' end and 14 bp at the 3' end. This peculiarity is shared by all members of the O-type subfamily. Comparison with other P element sequences indicates incongruities between the phylogenies of the species and the P transposons. M-type and O-type elements apparently have no common origin in the *D. bifasciata* lineage. The M-type sequence seems to be most closely related to the P element from *Scaptomyza pallida* and thus could be considered as a more recent invader of the *D. bifasciata* gene pool. The origin of the O-type elements cannot be unequivocally deduced from the present data.(ABSTRACT TRUNCATED AT 250 WORDS)

- **Handler, A. M., S. P. Gomez and D. A. O'Brochta.** 1993. Negative regulation of P element excision by the somatic product and terminal sequences of P in *Drosophila melanogaster*. *Mol. Gen. Genet.* **237**:145-151.

A transient in vivo P element excision assay was used to test the regulatory properties of putative repressor-encoding plasmids in *Drosophila melanogaster* embryos. The somatic expression of an unmodified transposase transcription unit under the control of a heat shock gene promoter (*phs pi*) effectively repressed P excision in a dose-dependent manner at very low concentrations relative to somatically active transposase (encoded by the *hs pi 2-3* gene). Maximum repression required transcription of the complete transposase gene. Dose-dependent repression of P excision was also observed in the presence of a vector plasmid (*pCarnegie4*) having only the terminal sequences, including transposase binding sites, of the P element. However, repression required considerably

higher concentrations of pCarnegie4 than phs pi, and elimination of P excision was not observed.

- **Hartenstein, V. and Y.-N. Jan.** 1992. Studying *Drosophila* embryogenesis with P-*lacZ* enhancer trap lines. *Roux's Archives of Dev. Biol.* **201**:194-220.
- **Higuet, D., D. Anxolabéhère and D. Nouaud.** 1992. A particular P element insertion is correlated to the P-induced hybrid dysgenesis repression in *Drosophila melanogaster*. *Genet. Res. Camb.* **60**:15-24.
- **Hiraizumi, Y.** 1979. A new method to distinguish between meiotic and premeiotic recombinational events in *Drosophila melanogaster*. *Genetics* **92**:543-554.
- **Houck, M. A., J. B. Clark, K. R. Peterson and M. G. Kidwell.** 1991. Possible horizontal transfer of *Drosophila* genes by the mite *Proctolaelaps regalis*. *Science* **253**:1125-1128.
- **Jackson, M. S., D. M. Black and G. A. Dover.** 1988. Amplification of *KP* elements associated with the repression of hybrid dysgenesis in *Drosophila melanogaster*. *Genetics* **120**:1003-1013.
- **Johnson, C. W.** 1913. The distribution of some species of *Drosophila*. *Psyche* **20**:202-204.
- **Johnson-Schlitz, D. M. and W. R. Engels.** 1993. P element-induced interallelic gene conversion of insertions and deletions in *Drosophila*. *Mol. Cell. Biol.* **13**:7006-7018.

We studied the process by which *whd*, a P element insertion allele of the *Drosophila white* locus, is replaced by its homolog in the presence of transposase. These events are interpreted as the result of double-strand gap repair following excision of the P transposon in *whd*. As templates for this repair we used a series of alleles derived from *whd* through P element mobility. One group of them, referred to collectively as *whd-F*, carried fragments of the P element that had lost some of the sequences needed in *cis* for mobility. The other group, *whd-D*, had lost all of the P insert and carried a deletion of some of the flanking DNA from *white*. The average replacement frequency was 43% for *whd-F* alleles and 7% for the *whd-D* alleles. Some of the former were converted at frequencies exceeding 50%. Our data suggest that the high conversion frequencies for the *whd-F* templates can be attributed at least in part to an elevated efficiency of repair of unexpanded gaps, possibly due to the closer match between *whd-F* sequences and the unexpanded gap endpoints. In addition, we found that the gene substitutions were almost exclusively in the direction of *whd* being replaced by the *whd-F* or *whd-D* allele rather than the reverse. The template alleles were usually unaltered in the process. This asymmetry implies that the conversion process is unidirectional and that the P fragments are not good substrates for P element transposase. Our results help elucidate a highly efficient double-strand gap repair mechanism in *Drosophila* that can also be used for gene replacement procedures involving insertions and deletions. They also help explain the rapid spread of P elements in populations.

- **Kaiser, K. and S. Goodwin.** 1990. "Site-selected" transposon mutagenesis of *Drosophila*. *Proc. Nat. Acad. Sci. USA* **87**:1686-1690.
- **Karess, R. E. and G. M. Rubin.** 1984. Analysis of P transposable element functions in *Drosophila*. *Cell* **38**:135-146.
- **Kassis, J. A., E. Noll, E. P. VanSickle, W. F. Odenwald and N. Perrimon.** 1992. Altering the insertional specificity of a *Drosophila* transposable element. *Proc. Natl. Acad. Sci. U. S. A.* **89**:1919-1923.

Vectors derived from the *Drosophila* P element transposon are widely used to make transgenic *Drosophila*. Insertion of most P-element- derived vectors is nonrandom, but they exhibit a broad specificity of target sites. During experiments to identify *cis*-acting regulatory elements of the *Drosophila* segmentation gene engrailed, we identified a fragment of engrailed DNA that, when included within a P-element vector, strikingly alters the specificity of target sites. P-element vectors that contain this fragment of

engrailed regulatory DNA insert at a high frequency near genes expressed in stripes.

- **Kaufman, P. D., R. F. Doll and D. C. Rio.** 1989. Drosophila P element transposase recognizes internal P element DNA sequences. *Cell* **59**:359-371.

- **Kaufman, P. D. and D. C. Rio.** 1991. Drosophila P-element transposase is a transcriptional repressor in vitro. *Proc. Natl. Acad. Sci. U. S. A.* **88**:2613-2617.

Mobility of P transposable elements in *Drosophila melanogaster* depends on the 87-kDa transposase protein encoded by the P element. Transposase recognizes a 10-base-pair DNA sequence that overlaps an A + T-rich region essential for transcription from the P-element promoter. We report here that transposase represses transcription from the P-element promoter in vitro. This transcriptional repression is blocked by prior formation of an RNA polymerase II transcription complex on the template DNA. Binding of transposase on the P-element promoter is blocked by prior binding of either the *Drosophila* RNA polymerase II complex or the yeast transcription factor TFIID. These data suggest that transposase represses transcription by preventing assembly of an RNA polymerase II complex at the P-element promoter.

- **Kaufman, P. D. and D. C. Rio.** 1992. P element transposition in vitro proceeds by a cut-and-paste mechanism and uses GTP as a cofactor. *Cell.* **69**:27-39.

We have developed an in vitro reaction system for *Drosophila* P element transposition. Transposition products were recovered by selection in *E. coli*, and contained simple P element insertions flanked by 8 bp target site duplications as observed in vivo.

Transposition required Mg²⁺ and partially purified P element transposase. Unlike other DNA rearrangement reactions, P element transposition in vitro used GTP as a cofactor; deoxyGTP, dideoxyGTP, or the nonhydrolyzable GTP analogs GMP-PNP or GMP-PCP were also used. Transposon DNA molecules cleaved at the P element termini were able to transpose, but those lacking 3'-hydroxyl groups were inactive. These biochemical data are consistent with genetic data suggesting that P element transposition occurs via a "cut-and-paste" mechanism.

- **Kelley, M. R., S. Kidd, R. L. Berg and M. W. Young.** 1987. Restriction of P element insertions at the *Notch* locus of *Drosophila melanogaster*. *Mol. Cell Biol.* **7**:1545-1548.
- **Kidwell, M. G.** 1979. Hybrid dysgenesis in *Drosophila melanogaster*: The relationship between the P-M and I-R interaction systems. *Genet. Res. Camb.* **33**:105-117.
- **Kidwell, M. G.** 1983. Evolution of hybrid dysgenesis determinants in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci. USA* **80**:1655-1659.
- **Kidwell, M. G.** 1986. P-M mutagenesis. pp. 59-82 in *Drosophila: A Practical Approach*, edited by D. B. Roberts. IRL Press, Oxford.
- **Kidwell, M. G.** 1993. Horizontal transfer of P elements and other short inverted repeat transposons. pp. in *Transposable Elements and Evolution*, edited by J. F. McDonald. Kluwer Academic Publishers, London.
- **Kidwell, M. G., T. Frydryk and J. B. Novy.** 1983. The hybrid dysgenesis potential of *Drosophila melanogaster* strains of diverse temporal and geographical natural origins. *Dros. Inform. Serv.* **59**:63-69.
- **Kidwell, M. G., J. F. Kidwell and J. A. Sved.** 1977. Hybrid dysgenesis in *Drosophila melanogaster*: A syndrome of aberrant traits including mutation, sterility, and male recombination. *Genetics* **86**:813-833.
- **Kidwell, M. G. and J. B. Novy.** 1979. Hybrid dysgenesis in *Drosophila melanogaster*: sterility resulting from gonadal dysgenesis in the P-M system. *Genetics* **92**:1127-1140.
- **Kiyasu, P. K. and M. G. Kidwell.** 1984. Hybrid dysgenesis in *Drosophila melanogaster*: the evolution of mixed P and M populations maintained at high

- temperature. *Genet. Res. Camb.* **44**:251-259.
- **Lachaise, D., M. L. Cariou, J. R. David, F. Lemeunier, L. Tsacas and M. Ashburner.** 1988. Historical biogeography of the *Drosophila melanogaster* species subgroup. *Evol. Biol.* **22**:159-225.
 - **Lankenau, D., V. Corces and W. R. Engels.** 1996. Comparison of targeted gene replacement-frequencies in *Drosophila* at the *forked* and *white* loci. *Mol. Cel. Biol.* **16**:3535-3544.

P element induced gene conversion has been previously used to modify the *white* gene of *Drosophila* in a directed fashion. The applicability of this approach of gene targeting in *Drosophila*, however, has not been analyzed quantitatively for other genes. We took advantage of the P-induced *forked* allele, *fhd*, which was used as a target, and we constructed a vector containing a modified *forked* fragment for converting *fhd*. Conversion frequencies were analyzed for this locus as well as for an alternative *white* allele, *weh812*. Combination of both P induced mutant genes allowed the simultaneous analysis of conversion frequencies under identical genetic, developmental and environmental conditions. This paper demonstrates that gene conversion through P-induced gap repair can be applied with similar success-rates at the *forked* locus as at the *white* gene. The average conversion frequencies at *forked* were 0.29% and at *white* 0.16%. These frequencies indicate that in vivo gene targeting in *Drosophila* should be applicable for other genes in this species at manageable rates. A different experiment revealed evidence that may indicate that a protein (*Su(Hw)*), which imparts control on chromatin-condensation, may interfere with the gap repair process.

- **Lansman, R. A., R. O. Shade, T. A. Grigliatti and H. W. Brock.** 1987. Evolution of P transposable elements: Sequences of *Drosophila nebulosa* P elements. *Proc. Natl. Acad. Sci. USA* **84**:6491-6495.
- **Lansman, R. A., S. N. Stacey, T. A. Grigliatti and H. W. Brock.** 1985. Sequences homologous to the P mobile element of *Drosophila melanogaster* are widely distributed in the subgenus *Sophophora*. *Nature* **318**:561-563.
- **Laski, F. A., D. C. Rio and G. M. Rubin.** 1986. Tissue specificity of *Drosophila* P element transposition is regulated at the level of mRNA splicing. *Cell* **44**:7-19.
- **Lemaitre, B. and D. Coen.** 1991. P regulatory products repress in vivo the P promoter activity in P-lacZ fusion genes. *Proc. Natl. Acad. Sci. U. S. A.* **88**:4419-4423.

The transposition of P elements in *Drosophila melanogaster* is regulated by products encoded by the P elements themselves. The molecular mechanisms of this regulation are complex and still unclear. We have assayed in vivo the effects of P regulatory products on the P promoter itself by using P-lacZ fusion genes. We have found that all the P-lacZ insertions are repressed in a P background. This repression occurs in all the tissues observed and at all the developmental stages. The amount of transcripts specific for P-lacZ is substantially reduced in a P background. These results suggest that P trans-acting products can exert a direct repression on the P promoter transcription.

- **Lemaitre, B., S. Ronsseray and D. Coen.** 1993. Maternal repression of the P element promoter in the germline of *Drosophila melanogaster*: a model for the P cytotype. *Genetics.* **135**:149-160.

The transposition of P elements in *Drosophila melanogaster* is regulated by products encoded by the P elements themselves. The P cytotype, which represses transposition and associated phenomena, exhibits both a maternal effect and maternal inheritance. The genetic and molecular mechanisms of this regulation are complex and not yet fully understood. In a previous study, using P-lacZ fusion genes, we have shown that P element regulatory products were able to inhibit the activity of the P promoter in somatic tissues. However, the repression observed did not exhibit the maternal effect characteristic of the P cytotype. With a similar approach, we have assayed in vivo the

effect of P element regulatory products in the germline. We show that the P cytotype is able to repress the P promoter in the germline as well as in the soma. Furthermore, this repression exhibits a maternal effect restricted to the germline. On the basis of these new observations, we propose a model for the mechanism of P cytotype repression and its maternal inheritance.

- **McDonald, J. F.** 1993. *Transposable Elements and Evolution*. Kluwer Academic Publishers, London
- **Meister, G. A. and T. A. Grigliatti.** 1993. Rapid spread of a P element/Adh gene construct through experimental populations of *Drosophila melanogaster*. *Genome* **36**:1169-75.

Transposable elements may be potential tools for the dispersal of engineered DNA through target insect populations. The utility of this hypothesis is predicted on the ability of transposable elements carrying a large DNA insert to rapidly disperse through a population. In addition, the inserted DNA must be replicated with a high degree of fidelity during this dispersal. We have monitored the ability of a transposable element with an inserted gene to spread through experimental populations and tested whether the passenger gene retains its ability to encode an active protein. Several *Drosophila melanogaster* laboratory populations were initiated with female flies that were null for alcohol dehydrogenase activity and contained no P elements. Most of the females were mated to males of the same strain; however, 1 or 10% of the females were mated to males from a strain that had previously been transformed with a helper P element and a P element/Adh gene construct. The dispersal of P elements to new genomes was monitored at each generation by randomly selecting females and performing DNA hybridization assays on dissected ovarian tissue. In addition, each female was tested for alcohol dehydrogenase activity using a simple histochemical assay. We find that, despite an approximate threefold increase in size, the P element constructs containing a functioning gene are still capable of rapid dispersal through the experimental populations. We also show that many of the inserted Adh genes still encode an active product.

- **Miller, W. J., S. Hagemann, E. Reiter and W. Pinsker.** 1992. P element-homologous sequences are tandemly repeated in the genome of *Drosophila guanche*. *Proc. Natl. Acad. Sci.* **89**:4018-4022.
- **Misra, S., R. M. Buratowski, T. Ohkawa and D. C. Rio.** 1993. Cytotype control of *Drosophila melanogaster* P element transposition: genomic position determines maternal repression. *Genetics* **135**:785-800.

P element transposition in *Drosophila* is controlled by the cytotype regulatory state: in P cytotype, transposition is repressed, whereas in M cytotype, transposition can occur. P cytotype is determined by a combination of maternally inherited factors and chromosomal P elements in the zygote. Transformant strains containing single elements that encoded the 66-kD P element protein zygotically repressed transposition, but did not display the maternal repression characteristic of P cytotype. Upon mobilization to new genomic positions, some of these repressor elements showed significant maternal repression of transposition in genetic assays, involving a true maternal effect. Thus, the genomic position of repressor elements can determine the maternal vs. zygotic inheritance of P cytotype. Immunoblotting experiments indicate that this genomic position effect does not operate solely by controlling the expression level of the 66-kD repressor protein during oogenesis. Likewise, P element derivatives containing the hsp26 maternal regulator sequence expressed high levels of the 66-kD protein during oogenesis, but showed no detectable maternal repression. These data suggest that the location of a repressor element in the genome may determine maternal inheritance of P cytotype by a mechanism involving more than the overall level of expression of the 66-kD protein in the ovary.

- **Misra, S. and D. C. Rio.** 1990. Cytotype control of *Drosophila* P element transposition: the 66 kd protein is a repressor of transposase activity. *Cell* **62**:269-284.
- **Mullins, M. C., D. C. Rio and G. M. Rubin.** 1989. *Cis*-acting DNA sequence requirements for P element transposition. *Genes and Development* **3**:729-738.
- **Nassif, N. A. and W. R. Engels.** 1993. DNA homology requirements for mitotic gap repair in *Drosophila*. *Proc. Nat. Acad. Sci. USA* **90**:1262-1266.

We used P transposable element mobilization to study the repair of double-strand DNA breaks in *Drosophila melanogaster* premeiotic germ cells. The distribution of conversion tracts was found to be largely unaffected by changes in the length of sequence homology between the broken ends and the template, suggesting that only a short match is required. However, the frequency of repair was highly sensitive to single-base mismatches within the homologous region, ranging from 19% reversion when there were no mismatches to 5% when 15 mismatches were present over a 3455 base-pair span.

- **Nassif, N. A., J. Penney, S. Pal, W. R. Engels and G. B. Gloor.** 1994. Efficient copying of nonhomologous sequences from ectopic sites via P element-induced gap repair. *Mol. Cell. Biol.* **14**:1613-1625.

P element-induced gap repair was used to copy nonhomologous DNA into the *Drosophila white* locus. We found that nearly 8000 base pairs of nonhomologous sequence could be copied in from an ectopic template at essentially the same rate as a single base substitution at the same location. An in vitro-constructed deletion was also copied into *white* at high frequencies. This procedure can be applied to the study of gene expression in *Drosophila*, especially for genes too large to be manipulated in other ways. We also observed several types of more complex events in which the copied template sequences were rearranged such that the breakpoints occurred at direct duplications. Most of these can be explained by a model of double strand break repair in which each terminus of the break invades a template independently and serves as a primer for DNA synthesis from it, yielding two overlapping single-stranded sequences. These single strands then pair, and synthesis is completed by each using the other as template. This synthesis-dependent strand annealing (SDSA) model is discussed as a possible general mechanism in complex organisms.

- **Niki, Y.** 1986. Germline autonomous sterility of P-M dysgenic hybrids and their application to germline transfers in *Drosophila melanogaster*. *Dev. Biol.* **113**:255-258.
- **Niki, Y. and S. I. Chigusa.** 1986. Developmental analysis of the gonadal sterility of P-M hybrid dysgenesis in *Drosophila melanogaster*. *Jpn. J. Genet.* **61**:147-156.
- **O'Brochta, D. A., S. P. Gomez and A. M. Handler.** 1991. P element excision in *Drosophila melanogaster* and related drosophilids. *Mol. Gen. Genet.* **225**:387-394.

The frequency of P element excision and the structure of the resulting excision products were determined in three drosophilid species. *Drosophila melanogaster*, *D. virilis*, and *Chymomyza procnemis*. A transient P element mobility assay was conducted in the cells of developing insect embryos, but unlike previous assays, this mobility assay permitted the recovery of excision products from plasmids regardless of whether the excision event was precise or imprecise. Both quantitative and qualitative differences between the products of excision in the various species studied were observed. The frequency with which P element excision products were recovered from *D. melanogaster* was 10-fold greater than from *D. virilis* and *C. procnemis*; however, the proportion of all excision events resulting in the reversion of a P-induced mutant phenotype was the same. Virtually all excision products recovered, including those resulting in a reversion of the mutant phenotype, did not result in the exact restoration of the original target sequence. Sequence analysis suggested that duplex cleavage at the 3' and 5' termini of the P element, or their subsequent modification, occurred asymmetrically and interdependently. P element-encoded transposase was not absolutely required for P element excision.

- **O'Hare, K., A. Driver, S. McGrath and D. M. Johnson-Schlitz.** 1992. Distribution and structure of cloned P elements from the *Drosophila melanogaster* P strain '2. *Genet. Res. Camb* **60**:33-41.

- **O'Hare, K. and G. M. Rubin.** 1983. Structure of P transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. *Cell* **34**:25-35.

We have isolated and characterized several members of the P transposable element family from a *Drosophila melanogaster* P strain. Large 2.9 kb elements are present as multiple highly conserved copies together with smaller (0.5-1.6 kb), heterogeneous elements. The complete DNA sequences of the 2.9 kb element and four small elements (previously isolated from hybrid-dysgenesis-induced mutations of the white locus) have been determined. Each small element appears to have arisen from the 2.9 kb element by a different internal deletion. P elements have 31 bp perfect inverse terminal repeats and upon insertion duplicate an 8 bp sequence found only once at the site of insertion. Three of the insertions into the white locus occurred at the same nucleotide, indicating a high degree of local site specificity for insertion. The basis of this specificity has been investigated by DNA sequence analysis of the sites where 18 P elements are found. A revertant of one of the white locus mutants has been found to result from precise excision of the P element, restoring the wild-type DNA sequence.

- **O'Kane, C. J. and W. J. Gehring.** 1987. Detection *in situ* of genomic regulatory elements in *Drosophila*. *Proc. Nat. Acad. Sci. USA* **84**:9123-9127.
- **Ochman, H., A. S. Gerber and D. L. Hartl.** 1988. Genetic applications of an inverse polymerase chain reaction. *Genetics* **120**:621-623.
- **Papoulas, O., K. McCall and W. Bender.** 1994. Targeted gene conversion at the bithorax complex. 35th Annual *Drosophila* Research Conference
- **Paques, F. and M. Wegnez.** 1993. Deletions and amplifications of tandemly arranged ribosomal 5S genes internal to a P element occur at a high rate in a dysgenic context. *Genetics*. **135**:469-476.

We observed unusual kinds of rearrangements within tandemly clustered 5S genes internal to a P element in dysgenic context. Rearranged P transposons, initially containing eight 5S genes, were found to display discrete numbers of 5S genes, from 4 up to 17 units. Precise deletions and amplifications occurred at a high rate (40%), at both original and new insertion sites. These events can be explained by a "cut and paste" transposition model. Possible links between rearrangements due to dysgenic-like processes and concerted evolution are discussed.

- **Paricio, N., M. Perez-Alonso, M. J. Martinez-Sebastian and R. de Frutos.** 1991. P sequences of *Drosophila subobscura* lack exon 3 and may encode a 66 kd repressor-like protein. *Nucleic. Acids. Res.* **19**:6713-6718.

Several P homologous sequences have been cloned and sequenced from *Drosophila subobscura*. These sequences are located at the 85DE region of the O chromosome and at least three of them are organized in tandem. We have identified four copies which exhibit strong similarity between them. All of the isolated elements are truncated at the 5' and 3' ends. They have lost the inverted terminal repeats and exon 3, but maintain exons 0, 1 and 2. They are transcribed producing a polyadenylated RNA. The structure of these transcripts suggests that they are able to encode a 66 kd repressor-like protein, but not a functional transposase. We ask about the biological role of a potential repressor protein in this species.

- **Perkins, H. D. and A. J. Howells.** 1992. Genomic sequences with homology to the P element of *Drosophila melanogaster* occur in the blowfly *Lucilia cuprina*. *Proc Natl Acad Sci U S A* **89**:10753-7.

We have cloned two DNA elements (Lu-P1 and Lu-P2) from the Australian sheep

blowfly *Lucilia cuprina* that are similar to the transposable P element of *Drosophila melanogaster* in both structure and sequence but have diverged from it and from each other considerably. Hybridization studies indicate that a third related element probably exists in another, as yet unsequenced, clone. Neither Lu-P1 nor Lu-P2 appears to be active in terms of mobility, and it is not known whether any transposition-competent copies of other related elements occur in the genome of the blowfly. However, the isolation of any P-like sequences from a species outside of the family Drosophilidae allows comparisons to be made of more widely divergent P-related elements than has been possible previously. We are unaware of any report of the presence of multiple P-like family members within a single species. The discovery of Lu-P1 and Lu-P2 in the blowfly fuels the possibility that similar elements may be widespread in insects, and perhaps in other orders of animals.

- **Pirrotta, V.** 1988. Vectors for P-mediated transformation in *Drosophila*. pp. 437-456 in *Vectors, A Survey of Molecular Cloning Vectors and their Uses*, edited by R. L. Rodriguez and D. T. Denhardt. Butterworths, London.
- **Preston, C. R. and W. R. Engels.** 1989. Spread of P transposable elements in inbred lines of *Drosophila melanogaster*. pp. 71-85 in *Progress in Nucleic Acid Research and Molecular Biology: Hollaender Symposium Proceedings*, edited by W. Cohn and K. Moldave. Academic Press, San Diego.

The invasion of the *Drosophila melanogaster* genome by members of the P family of transposable elements was monitored by in situ hybridization to polytene chromosomes. Populations consisted of inbred lines starting with a single element. There were several cases of very rapid proliferation of element copy numbers, going from a single copy to more than twenty in the span of a few generations. These episodes, which occurred during periods of intense inbreeding and usually led to extinction of the stock, were preceded by 6-20 generations during which the copy number remained at one. In other cases, the founding P element was lost before any proliferation could occur, and in still others the stock gradually developed the "P cytotype" in which P element mobility is suppressed. Finally, there was one transposase-producing P element that appeared to be stable or nearly so. Possible explanations for this behavior and implications for natural populations are discussed.

- **Preston, C. R. and W. R. Engels.** 1996. P element-induced male recombination and gene conversion in *Drosophila*. *Genetics* (**in press**)

A P element insertion flanked by 13 RFLP marker sites was used to examine male recombination and gene conversion at an autosomal site. The great majority of crossovers on chromosome arm 2R occurred within the 4-kb region containing the P element and RFLP sites. Of the 128 recombinants analyzed, approximately two-thirds carried duplications or deletions flanking the P element. These rearrangements are described in more detail in the accompanying report. In a parallel experiment, we examined 91 gene conversion tracts resulting from excision of the same autosomal P element. We found the average tract length was 1463 bp, which is essentially the same as found previously at the white locus. The distribution of conversion tract endpoints was indistinguishable from the distribution of crossover points among the non rearranged male recombinants. Most recombination events can be explained by the "hybrid element insertion" model, but, for those lacking a duplication or deletion, a second step involving double strand gap repair must be postulated to explain the distribution of crossover points.

(Accompanying papers by [Gray, Tanaka & Sved](#), and [Preston, Sved & Engels](#))

- **Preston, C. R., J. A. Sved and W. R. Engels.** 1996. Flanking duplications and deletions associated with P-induced male recombination in *Drosophila*. *Genetics* (**in press**)

We studied P element-induced recombination in germline mitotic cells by examining the

structure of the recombinant chromosomes. We found that most recombinants retain a mobile *P* element at the site of the recombination, usually with either a deletion or a duplication immediately adjacent to the *P* end at which the crossover occurred. The sizes of these deletions and duplications ranged from a few base pairs to well over 100 kb. These structures fit the "hybrid element insertion" (HEI) model of male recombination in which the two *P* element copies on sister chromatids combine to form a "hybrid element" whose termini insert into a nearby position on the homolog. The data suggest that *P*-induced recombination can be used as an efficient means of generating flanking deletions in the vicinity of existing *P* elements. These deletions are easily screened using distant flanking markers, and they can be chosen to extend in a given direction depending on which reciprocal recombinant type is selected. Furthermore, the retention of a mobile *P* element allows one to extend the deletion or generate additional variability at the site by subsequent rounds of recombination.

(Accompanying papers by [Gray, Tanaka & Sved](#), and [Preston & Engels](#))

- **Rasmusson, K. E., J. D. Raymond and M. J. Simmons.** 1993. Repression of hybrid dysgenesis in *Drosophila melanogaster* by individual naturally occurring P elements. *Genetics*. **133**:605-622.

Individual P elements that were genetically isolated from wild-type strains were tested for their abilities to repress two aspects of hybrid dysgenesis: gonadal dysgenesis and mutability of a double-P element-insertion allele of the singed locus (*snw*). These elements were also characterized by Southern blotting, polymerase chain reaction amplification and DNA sequencing. Three of the elements were 1.1-kb KP elements, one was a 1.2-kb element called D50, and one was a 0.5-kb element called SP. These three types of elements could encode polypeptides of 207, 204, and 14 amino acids, respectively. Gonadal dysgenesis was repressed by two of the KP elements (denoted KP(1) and KP(6)) and by SP, but not by the third KP element (KP(D)), nor by D50. Repression of gonadal dysgenesis was mediated by a maternal effect, or by a combination of zygotic and maternal effects generated by the P elements themselves. The mutability of *snw* was repressed by the KP(1) and KP(6) elements, by D50 and by SP, but not by KP(D); however, the SP element repressed *snw* mutability only when the transposase came from complete P elements and the D50 element repressed it only when the transposase came from the modified P element known as 2-3. In all cases, repression of *snw* mutability appeared to be mediated by a zygotic effect of the isolated P element. Each of the isolated elements was also tested for its ability to suppress the phenotype of a P-insertion mutation of the vestigial locus (*vg21-3*). D50 was a moderate suppressor whereas SP and the three KP elements had little or no effect. These results indicate that each isolated P element had its own profile of repression and suppression abilities. It is suggested that these abilities may be mediated by P-encoded polypeptides or by antisense P RNAs initiated from external genomic promoters.

- **Raymond, J. D., T. A. Ojala, J. White and M. J. Simmons.** 1991. Inheritance of P-element regulation in *Drosophila melanogaster*. *Genet. Res.* **57**:227-234.

The ability to repress P-element-induced gonadal dysgenesis was studied in 14 wild-type strains of *D. melanogaster* derived from populations in the central and eastern United States. Females from each of these strains had a high ability to repress gonadal dysgenesis in their daughters. Reciprocal hybrids produced by crossing each of the wild-type strains with an M strain demonstrated that repression ability was determined by a complex mixture of chromosomal and cytoplasmic factors. Cytoplasmic transmission of repression ability was observed in all 14 strains and chromosomal transmission was observed in 12 of them. Genomic Southern blots indicated that four of the strains possessed a particular type of P element, called KP, which has been proposed to account for the chromosomal transmission of repression ability. However, in this study several of the strains that lacked KP elements exhibited as much chromosomal transmission of

repression ability as the strains that had KP elements, suggesting that other kinds of P elements may be involved.

- **Rio, D. C.** 1990. Molecular mechanisms regulating *Drosophila* P element transposition. *Annu. Rev. Genet.* **24**:543-578.
- **Rio, D. C., F. A. Laski and G. M. Rubin.** 1986. Identification and immunochemical analysis of biologically active *Drosophila* P element transposase. *Cell* **44**:21-32.
- **Rio, D. C. and G. M. Rubin.** 1988. Identification and purification of a *Drosophila* protein that binds to the terminal 31-base-pair repeats of the P transposable element. *Proc. Natl. Acad. Sci. USA* **85**:8929-8933.
- **Robertson, H. M.** 1993. The mariner transposable element is widespread in insects [see comments]. *Nature* **362**:241-245.

The mariner transposable element is a small member of the short inverted terminal repeat class thought to transpose through a DNA intermediate. Originally described in *Drosophila mauritiana*, it is now known in several species of the family Drosophilidae, and in a moth *Hyalophora cecropia*. Here I use primers designed to represent regions of amino-acid conservation between the putative transposase genes of the *D. mauritiana* and *H. cecropia* elements to amplify equivalent regions of presumed mariner elements from ten other insects representing six additional orders, including the malaria- vector mosquito, *Anopheles gambiae*. Sequences of multiple clones from each species reveal a diverse array of mariner elements, with multiple subfamilies in the genomes of some insects, indicating both vertical inheritance and horizontal transfers. An intact open reading frame in at least one clone from each species suggests each may carry functional transposable elements. Therefore the mariner element is an excellent candidate for development of genetic transformation systems for non-drosophilid insects, and possibly other arthropods.

- **Robertson, H. M. and W. R. Engels.** 1989. Modified P elements that mimic the P cytotype in *Drosophila melanogaster*. *Genetics* **123**:815-823.

Activity of the P family of transposable elements in *Drosophila melanogaster* is regulated primarily by a cellular condition known as P cytotype. It has been hypothesized that P cytotype depends on a P element-encoded repressor of transposition and excision. We provide evidence in support of this idea by showing that two modified P elements, each with lesions affecting the fourth transposase exon, mimic most of the P cytotype effects. These elements were identified by means of two sensitive assays capable of detecting repression by a single P element. One assay makes use of cytotype-dependent gene expression of certain P element insertion mutations at the singed bristle locus. The other measures suppression of transposase activity from the unusually stable genomic P element, 2-3(99B), that normally produces transposase in both germinal and somatic tissues. The P cytotype-like effects include suppression of *snw* germline hypermutability, *snw* somatic mosaicism, pupal lethality, and gonadal dysgenic sterility. Unlike P cytotype, however, there was no reciprocal cross effect in the inheritance of repression.

- **Robertson, H. M., C. R. Preston, R. W. Phillis, D. Johnson-Schlitz, W. K. Benz and W. R. Engels.** 1988. A stable genomic source of P element transposase in *Drosophila melanogaster*. *Genetics* **118**:461-470.

A single P element insert in *Drosophila melanogaster*, called P[ry+ 2-3](99B), is described that caused mobilization of other elements at unusually high frequencies, yet is itself remarkably stable. Its transposase activity is higher than that of an entire P strain, but it rarely undergoes internal deletion, excision or transposition. This element was constructed by F. Laski, D. Rio and G. Rubin for other purposes, but we have found it to be useful for experiments involving P elements. We demonstrate that together with a chromosome bearing numerous nonautonomous elements it can be used for P element mutagenesis. It can also substitute efficiently for "helper" plasmids in P element mediated

transformation, and can be used to move transformed elements around the genome.

- **Roiha, H., G. M. Rubin and K. O'Hare.** 1988. P element insertions and rearrangements at the *singed* locus of *Drosophila melanogaster*. *Genetics* **119**:75-83.
- **Ronsseray, S., M. Lehmann and D. Anxolabéhère.** 1991. The maternally inherited regulation of P elements in *Drosophila melanogaster* can be elicited by two P copies at cytological site 1A on the X chromosome. *Genetics*. **129**:501-512.

Two P elements, inserted at the cytological site 1A on an X chromosome from an *Drosophila melanogaster* natural population (Lerik, USSR), were isolated by genetic methods to determine if they are sufficient to cause the P cytotype, the cellular condition that regulates the P family of transposable element. The resulting "Lerik P(1A)" line (abbreviated "Lk-P(1A)") carries only one P element in situ hybridization site but genomic Southern analysis indicates that this site contains two, probably full length, P copies separated by at least one EcoRI cleavage site. Because the Lk-P(1A) line shows some transposase activity, at least one of these two P elements is autonomous. The Lk-P(1A) line fully represses germline P element activity as judged by the GD sterility and *snw* hypermutability assays; this result shows that the P cytotype can be elicited by only two P element copies. However, the Lk-P(1A) line does not fully repress 2-3(99B) transposase activity in the soma, although it fully represses 2-3(99B) transposase activity in the germline (2-3(99B) is an in vitro modified P element that produces a high level of transposase activity in both the germline and the soma). The germline regulatory properties of the Lk-P(1A) line are maternally transmitted, even when the 2-3(99B) element is used as the source of transposase. By contrast, the partial regulation of 2-3(99B) somatic activity is chromosomally inherited. These results suggest that the regulatory P elements of the Lk-P(1A) line are inserted near a germline-specific enhancer.

- **Ronsseray, S., B. Lemaitre and D. Coen.** 1993. Maternal inheritance of P cytotype in *Drosophila melanogaster*: a "pre- P cytotype" is strictly extra-chromosomally transmitted. *Mol. Gen. Genet.* **241**:115-123.

In *Drosophila melanogaster*, transposition of the P element is under the control of a cellular state known as cytotype. The P cytotype represses P transposition whereas the M cytotype is permissive for transposition. In the long-term, the P cytotype is determined by chromosomal P elements but over a small number of generations it is maternally inherited. In order to analyse the nature of this maternal inheritance, we tested whether a maternal component can be transmitted without chromosomal P elements. We used a stable determinant of P cytotype, linked to the presence of two P elements at the tip of the X chromosome (1A site) in a genome devoid of other P elements. We measured P repression capacity using two different assays: gonadal dysgenic sterility (GD) and P-lacZ transgene repression. We show that zygotes derived from a P cytotype female (heterozygous for P (1A)/balancer devoid of P copies) and which inherit no chromosomal P elements from the mother, have, however, maternally received a P-type extra-chromosomal component: this component is insufficient to specify the P cytotype if the zygote formed does not carry chromosomal P elements but can promote P cytotype determination if regulatory P elements have been introduced paternally. We refer to this strictly extra-chromosomally inherited state as the "pre-P cytotype". In addition, we show that a zygote that has the pre-P cytotype but which has not inherited any chromosomal P elements, does not transmit the pre-P cytotype to the following generation. The nature of the molecular determinants of the pre-P cytotype is discussed.

- **Rubin, G. M., M. G. Kidwell and P. M. Bingham.** 1982. The molecular basis of P-M hybrid dysgenesis: The nature of induced mutations. *Cell* **29**:987-994.
- **Rubin, G. M. and A. C. Spradling.** 1982. Genetic transformation of *Drosophila* with transposable element vectors. *Science* **218**:348-353.

- **Salz, H. K., T. W. Cline and P. Schedl.** 1987. Functional changes associated with structural alterations induced by mobilization of a P element inserted in the *Sex-lethal* gene of *Drosophila*. *Genetics* **117**:221-231.
- **Searles, L. L., R. S. Jokerst, P. M. Bingham, R. A. Voelker and A. L. Greenleaf.** 1982. Molecular cloning of sequences from a *Drosophila RNA polymerase II* locus by P element transposon tagging. *Cell* **31**:585-592.
- **Sentry, J. W. and K. Kaiser.** 1994. Application of inverse PCR to site-selected mutagenesis of *Drosophila*. *Nucleic Acids Res* **22**:3429-30.
- **Serano, T. L., H. K. Cheung, L. H. Frank and R. S. Cohen.** 1994. P element transformation vectors for studying *Drosophila melanogaster* oogenesis and early embryogenesis. *Gene*. **138**:181-186.

We have constructed six new P-element-based *Drosophila melanogaster* transformation vectors that specifically allow for the high-level accumulation of any RNA of interest in the developing egg and pre- blastoderm embryo. Such specificity results, in part, from the inclusion in the vectors of an enhancer active exclusively in nurse cells, the principal providers of RNA to the egg and early embryo. The nurse cell enhancer was derived from the hsp26 heat-shock (HS) gene, but its activity was neither dependent on nor sensitive to HS. In addition to the nurse cell enhancer, two of the vectors contain sequences from the K10 gene that promote the early transfer of RNAs from nurse cells into the oocyte; RNAs that contain the K10 sequence are transferred into the oocyte during the early to middle stages of oogenesis (i.e., during stages 2-9), while RNAs that lack such sequences are stored in nurse cells until stage 11. All of the vectors contain a tsp and a multiple cloning site (MCS) immediately downstream from the hsp26 nurse cell enhancer. In three of the vectors, the MCS is preceded by an ATG start codon. A wild-type copy of the white gene is included in all of the vectors as a selectable marker for transformation. The specificity of the vectors was demonstrated by the analysis of the expression patterns of lacZ derivatives.

- **Siebel, C. W. and D. C. Rio.** 1990. Regulated splicing of the *Drosophila* P transposable element third intron in vitro: somatic repression. *Science* **248**:1200-1208.
- **Simmons, M. J., J. D. Raymond, N. Johnson and T. Fahey.** 1984. A comparison of mutation rates for specific loci and chromosome regions in dysgenic hybrid males of *Drosophila melanogaster*. *Genetics* **106**:85-94.
- **Simonelig, M. and D. Anxolabehere.** 1991. A P element of *Scaptomyza pallida* is active in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U. S. A.* **88**:6102-6106.

Several results suggest that P elements have recently invaded natural populations of *Drosophila melanogaster* after a horizontal transfer from another species. The donor species is thought to come from the willistoni group, which contains P elements very homologous to those of *D. melanogaster*. However, more divergent P elements are present in many other Drosophilidae species. We have analyzed such elements from *Scaptomyza pallida*, a species phylogenetically distant to *D. melanogaster*. We report here the isolation of two coding P elements from *S. pallida* (PS2 and PS18) that are 4% divergent from one another. At least one of these elements (PS18) is active since it is able to transpose in *D. melanogaster* and to mobilize a *D. melanogaster* defective P element, even though its nucleotide sequence is 24% divergent from the canonical P element of *D. melanogaster*. To our knowledge, a P element that is active and strongly divergent from the *D. melanogaster* P element has not been reported previously. Sequence comparison between the complete P elements of *D. melanogaster* and *S. pallida* reveals that the structural characteristics are maintained: PS2 and PS18 contain terminal inverted repeats and internal repeats very similar to those of the *D. melanogaster* P element. In addition, the noncoding regions cis necessary for the transposition are more conserved than the coding sequences. Two domains found in the *D. melanogaster* P transposase (helix-turn-helix and leucine zipper) are well conserved in the putative proteins encoded by PS2 and PS18. This study provides insights into which parts of P elements are functionally

important and correlates with functional studies of the P element in *D. melanogaster*.

- **Spradling, A. C.** 1986. P element-mediated transformation. pp. 175-197 in *Drosophila: A Practical Approach*, edited by D. B. Roberts. IRL Press, Oxford.
- **Spradling, A. C. and G. M. Rubin.** 1982. Transposition of cloned P elements into *Drosophila* germ line chromosomes. *Science* **218**:341-347.
- **Staveley, B. E., R. B. Hodgetts, S. L. O'Keefe and J. B. Bell.** 1994. Targeting of an enhancer trap to vestigial. *Dev Biol* **165**:290-3.

The vestigial gene (*vg+*) is required for normal wing development and is expressed in a spatially distinct pattern in imaginal discs. We have exploited a general property of P element alleles to target an enhancer trap to the 5' region of the gene. By replacing the P element resident at this site in *vg21* with a P element carrying a *lacZ* reporter gene, the *vglacZ1* allele was selected on the basis of its increased mutant phenotype. In contrast to *vg+* expression, which occurs primarily in the presumptive wing margin and hinge, beta-galactosidase expression in *vglacZ1* wing discs is localized to the dorsal wing surface and displays homologous haltere expression. The targeting of P element enhancer traps could be readily extended to other genes with low rates of primary P element insertion.

- **Sturtevant, A. H.** 1921. The North American Species Of *Drosophila*. *Carnegie Inst. Wash. Publ* **301**
- **Sved, J. A., L. M. Blackman, A. S. Gilchrist and W. R. Engels.** 1991. High levels of recombination induced by homologous P elements in *Drosophila melanogaster*. *Mol. Gen. Genet.* **225**:443-447.

P element transposons in *Drosophila melanogaster* are capable of mobilizing incomplete P elements elsewhere in the genome, and of inducing recombination. This recombination is usually only of the order of 1% or less. We show that two P elements, located at exactly homologous sites, induce levels of recombination of 20% or higher. The recombination appears to be exact, as determined by the lack of phenotypic effects in recombinant products and the lack of size changes detectable by Southern hybridization. Female recombination is increased, but to a lesser extent than male recombination. Somatic recombination levels are also elevated. Alternative explanations for the high recombination levels are given in terms of the consequences of repair of an excision site and in terms of recombination as part of the replicative transposition process.

- **Sved, J. A., W. B. Eggleston and W. R. Engels.** 1990. Germline and somatic recombination induced by *in vitro* modified P elements in *Drosophila melanogaster*. *Genetics* **124**:331-337.

The P element insertion 2-3(99B) has previously been shown to activate incomplete P elements elsewhere in the genome. We show that this element, in conjunction with a second incomplete P element, *P[CaSpeR]*, also induces recombination in the male germline. In the absence of the *P[CaSpeR]* element, 2-3(99B) induces recombination at a much lower level. The recombination is induced preferentially in the region of the *P[CaSpeR]* element. Recombinant chromosomes contain the *P[CaSpeR]* element in more than 50% of cases, and alternative models of transposon replication and preferential chromosome breakage are put forward to explain this finding. As is the case with male recombination induced by P-M dysgenic crosses, recombination appears to be pre-meiotic in a high proportion of cases. The 2-3(99B) element is known to act in somatic cells. Correspondingly, we show that the 2-3(99B) - *P[CaSpeR]* combination elevates the incidence of somatic recombination.

- **Svoboda, Y., M. Robson and J. Sved.** 1995. P element-induced male recombination can be produced in *Drosophila melanogaster* by combining end-deficient elements in *trans*. *Genetics* **139**:1601-1610.
- **Syvanen, M.** 1984. The evolutionary implications of mobile genetic elements. *Ann.*

Rev. Genet. **18**:271-293.

- **Takasu-Ishikawa, E., M. Yoshihara and Y. Hotta.** 1992. Extra sequences found at P element excision sites in *Drosophila melanogaster*. *Mol. Gen. Genet.* **232**:17-23. We have previously established a transgenic *Drosophila* line with a highly transposable P element insertion. Using this strain we analyzed transposition and excision of the P element at the molecular level. We examined sequences flanking the new insertion sites and those of the remnants after excision. Our results on mobilization of the P element demonstrate that target-site duplication at the original insertion site does not play a role in forward excision and transposition. After P element excision an 8 bp target-site duplication and part of the 31 bp terminal inverted repeat (5-18 bp) remained in all the strains examined. Moreover, in 11 out of 28 strains, extra sequences were found between the two remaining inverted repeats. The double-strand gap repair model does not explain the origin of these extra sequences. The mechanism creating them may be similar to the hairpin model proposed for the transposon Tam in *Antirrhinum majus*.

- **Tower, J., G. H. Karpen, N. Craig and A. C. Spradling.** 1993. Preferential transposition of *Drosophila* P elements to nearby chromosomal sites. *Genetics*. **133**:347-359.

Two different schemes were used to demonstrate that *Drosophila* P elements preferentially transpose into genomic regions close to their starting sites. A starting element with weak *rosy+* marker gene expression was mobilized from its location in the subtelomeric region of the 1,300-kb Dp1187 minichromosome. Among progeny lines with altered *rosy+* expression, a much higher than expected frequency contained new insertions on Dp1187. Terminal deficiencies were also recovered frequently. In a second screen, a *rosy(+)*-marked element causing a lethal mutation of the *cactus* gene was mobilized in male and female germlines, and viable revertant chromosomes were recovered that still contained a *rosy+* gene due to an intrachromosomal transposition. New transpositions recovered using both methods were mapped between 0 and 128 kb from the starting site. Our results suggested that some mechanism elevates the frequency 43-67-fold with which a P element inserts near its starting site. Local transposition is likely to be useful for enhancing the rate of insertional mutation within predetermined regions of the genome.

- **Tseng, J. C., S. Zollman, A. C. Chain and F. A. Laski.** 1991. Splicing of the *Drosophila* P element ORF2-ORF3 intron is inhibited in a human cell extract. *Mech. Dev.* **35**:65-72.

P element transposition in *Drosophila melanogaster* is regulated by germline-specific splicing of the P element ORF2-ORF3 intron. This regulation has been shown to depend on a cis-acting sequence located in the exon 12-31 bases from the 5' splice site. Mutations within this sequence disrupt the regulation and result in splicing of the ORF2-ORF3 intron in all tissues, indicating that the sequence is required to inhibit splicing of this intron in the soma. We now show that a trans-acting factor in a human (HeLa) cell extract can inhibit splicing of the intron, suggesting that this regulatory mechanism is conserved from flies to humans.

- **Tsubota, S., M. Ashburner and P. Schedl.** 1985. P element-induced control mutations at the *r* gene of *Drosophila melanogaster*. *Mol. Cell. Biol.* **5**:2567-2574.
- **Tsubota, S. and P. Schedl.** 1986. Hybrid dysgenesis-induced revertants of insertions at the 5' end of the *rudimentary* gene in *Drosophila melanogaster*: transposon-induced control mutations. *Genetics* **114**:165-182.
- **Wei, G., B. Oliver and A. P. Mahowald.** 1991. Gonadal dysgenesis reveals sexual dimorphism in the embryonic germline of *Drosophila* [published erratum appears in *Genetics* 1992 Jan;130(1):235]. *Genetics*. **129**:203-210.

In hybrid dysgenesis, sterility can occur in both males and females. At 27.5 degrees,

however, we found that P element-induced germline death was restricted to females. This sex-specific gonadal dysgenesis (GD) is complete by the first larval instar stage. As such, GD at 27.5 degrees reveals the sexually dimorphic character of the embryonic germline. The only other known dimorphic trait of the embryonic germline is the requirement for ovo. ovo is required for germline development in females only and has been implicated in germline sex determination. Dominant mutations of ovo partially suppressed female GD. Although embryonic germ cells are undifferentiated and morphologically indistinguishable between males and females, the functional dimorphism seen in ovo requirement and GD at 27.5 degrees indicates that sexual identity in *Drosophila* germ cells is established in embryogenesis.

- **Williams, J. A. and J. B. Bell.** 1988. Molecular organization of the vestigial region in *Drosophila melanogaster*. *EMBO J* **7**:1355-1363.

The vestigial (*vg*) locus of *Drosophila melanogaster* is involved in wing margin development. In the absence of a *vg+* gene, extensive cell death occurs in third instar imaginal discs which results in a complete loss of adult wing margin structures. P-element tagging was used to obtain a molecular clone of the *vg* locus, which led to the molecular characterization of approximately 46 kb of DNA from the region. Deficiency analysis and molecular mapping identified sequences, spanning approximately 20 kb of DNA within the larger region, which are necessary for *vg* function. The molecular map was oriented with respect to a pre-existing genetic fine structure map of the locus. The centromere distal limits of the locus were defined by deficiency analyses while the proximal end has not yet been conclusively established. However, three transcripts, that are apparently unrelated to *vg*, provide circumstantial evidence for the proximal limits of the *vg* locus. The nature of the molecular lesions for several extant recessive or lethal *vg* alleles was determined, and these were placed on the *vg* molecular map. The characterization of the lesions associated with two dominant *vg* alleles and one complex *vg* allele imply interesting regulatory mechanisms for this locus. As well, a revertant of a 412 insertion mutant allele was shown to have resulted from a further insertion of a roo element into the 412 element.

- **Williams, J. A., S. S. Pappu and J. B. Bell.** 1988. Suppressible P element alleles of the *vestigial* locus in *Drosophila melanogaster*. *Mol. Gen. Genet.* **212**:370-374.
- **Xu, T. and G. M. Rubin.** 1993. Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development.* **117**:1223-1237.

We have constructed a series of strains to facilitate the generation and analysis of clones of genetically distinct cells in developing and adult tissues of *Drosophila*. Each of these strains carries an FRT element, the target for the yeast FLP recombinase, near the base of a major chromosome arm, as well as a gratuitous cell-autonomous marker. Novel markers that carry epitope tags and that are localized to either the cell nucleus or cell membrane have been generated. As a demonstration of how these strains can be used to study a particular gene, we have analyzed the developmental role of the *Drosophila* EGF receptor homolog. Moreover, we have shown that these strains can be utilized to identify new mutations in mosaic animals in an efficient and unbiased way, thereby providing an unprecedented opportunity to perform systematic genetic screens for mutations affecting many biological processes.