THE POPULATION GENETICS OF THE P ELEMENT OF *DROSOPHILA MELANOGASTER*.

by

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ABSTRACT

The transposable P element of Drosophila melanogaster is known to cause sterility, high mutation rates and chromosome aberrations in crosses where the element transposes. P elements are absent from Drosophila strains collected over forty years ago, but are presently found in over 90% of wild-type populations. Given the detrimental effects P elements have on their host, population geneticists have been interested in how these elements have spread so rapidly in nature.

This study was undertaken in order to understand how the transposable P element can, on one hand, reduce the fitness of its host, and on the other hand appear to spread very rapidly through nature.

The ability of P elements to spread in natural populations was studied by setting up specific backcross experiments and population cage experiments which were designed to model the introduction of a small number of P-type flies into a large population of M-type flies (flies that lack the P element). In order to clearly demonstrate that the P element itself has increased in frequency, it was necessary to be able to identify (1) the number of P-type flies, as measured by gonadal dysgenesis and (2) the number of copies of P elements in the population. The P element copy number was measured using the technique of DNA hybridization.

In the first set of experiments, P-type flies were mated to M-type flies and the resulting progeny were repeatedly

backcrossed to M-type flies. It was shown that in these experiments, the P element activity (ie: the ability to induce gonadal dysgenesis) decreased with each subsequent generation of backcrossing. This decrease in P element activity was matched by an equivalent decrease in P element copy number. This experiment, which is equivalent to introducing P-type flies into an infinitely large M-type population, suggests that there are conditions under which P elements will be lost from a population.

The ability of P elements to spread in randomly mating populations was studied by setting up population cages with different P strains and several different starting frequencies (1% and 10%). The rate of increase of P-type flies in these cages was found to be extremely rapid, approaching 100% increase per generation in the initial generations (up to generation 5). By generation twenty, all of the population cages had at least 60% P-type flies. This increase in P-type flies was matched by a corresponding increase in the P element copy number in these cages. By using a marked X chromosome, it was also shown that the increase in P-type flies was unlikely to have occurred as a result of selection for P element carrying chromosomes. Thus P elements seem capable of spreading in randomly mating populations, presumably as a result of replicative transposition.

The differences between the backcross experiments and population cage experiments suggested a third set of experiments to elucidate these differences. These experiments were similar to the backcross experiments, however, a number of different

mating combinations were tested each generation. The P element activity of flies from a particular mating was found to be strongly affected by the ancestral genotype of those flies.

The results from this study and previous work strongly suggest that P elements can spread through natural populations as a result of their ability to transpose replicatively, even though they can have a negative effect on the fitness of their host. Several models for the spread of transposable elements have been proposed and even though these models have several limitations, they provide a fairly accurate description of the nature of P element spread. The evolution of self-regulated transposition is also discussed and it is pointed out how important this process is in preventing the over-replication of an element and the subsequent destruction of its host.

RESUME

L'élément transposable P provoque la stérilité, taux de mutation élevé et aberrations chromosomiques chez Drosophila melanogaster. Les souches de Drosophiles récoltées il y a plus de 40 ans ne contiennent pas d'éléments P, alors que l'on en trouve dans plus 90% des populations actuelles. Les généticiens de populations s'intéressent aux mécanismes par lesquels ces éléments P, bien que très délétères, se sont repandus si rapidement dans les populations naturelles. L'étude présentée ici tente de determiner comment la fréquence de l'élément transposable P peut se maintenir ou augmenter dans les populations naturelles.

La discrimination de l'élément P dans les populations naturelles a été étudiée à l'aide d'expériences de backcross et de cage à population dans lesquelles l'introduction d'un petit nombre de mouches de type P dans une population de mouches de type M (c'ést à dire ne portant pas l'élément P) a ete simulee. Pour determiner si l'augmentation de frequence de l'element P est le resultat de la transposition, il faut pouvoir dénombrer d'une part les mouches de type P, et d'autre part les copies de l'élément P dans la population; la me sure de la dysgénésis gonadique (c'est à dire de la sterilité induite) et l'hybridization de l'ADN ont été respectivement utilisées. Dans une première serie d'expériences de mouches de type P ont été croisés à des mouches de type M, leur descendance étant croisée en retour pendant plusieurs générations successives à des mouches

de type M: l'activitè de l'élément P (c'est à dire la capacité à provoquer la dysgenesis gonadique) diminue de backcross en backcross. Une diminution du nombre de copies de l'élément P accompagne cette baisse d'activite. Dans certaines conditions les elements P peuvent donc être éliminés d'une population.

La dissemination des éléments P dans des populations panmictiques a été étudiée à l'aide de cages à populations constituées à partir de souches P d'origines geographique différents, leur fréquence initiale etant ou 10%. L'augementation de fréquence des mouches de type P dans ces cages a été extrèmement rapide, proche de 100% par génération. À la 20^{e} génération, 60% des mouches de chaques cages étaient de type P. Parallèlement le nombre de copies de l'élément P a augmenté dans ces cages. L'utilisation d'un chromosome X marque a permis de dèmontrer que cette augmentation de nombre de mouches de type P ne rèsulte pas d'une selection des chromosomes porteurs de l'element P. Les élément P semblent donc capables de se disseminer dans des populations panmictiques par transposition rèplicative.

Une troisième sèries d'expériences a été entreprise pour expliquer les resultats contradictoires des expériences de backcross et des évolutions de fréquences en cages à population. Le principe de croisements utilisé dans la première expérience a été appliqué à différents types de croisements, ce qui a permis de montrer que l'activité de l'élément P des mouches issues d'un croisement donné depend du génotype des parents. Les resultats

de ce travail confirment des travaux anterieures et suggèrent que les elements P peuvent se répandre dans les populations naturelles par transposition replicative, en depit de leur effet negatif sur la valeur adaptive de leur hôte. Plusieurs modèles ont été proposés pour expliquer cette dissèmination des éléments transposable: à quelques restrictions pres, ils ne sont pas contredits par les resultats obtenus ici. L'évolution de la transposition vers une auto-régulation est discutée, et l'importance de ce processus pour éviter toute réplication excessive est soulignée, un tel excès ne pouvant aboutir qu,à la disparition de la population hôte.

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CHAPTER 1

INTRODUCTION

Introduction:

Until recently, geneticists have viewed the genome as being relatively stable, subject to slow evolutionary change. This stable behaviour of Mendelian factors was important in permitting the construction of genetic maps and was further emphasized by the similarity in genome organization between closely related species (Lewin, 1983). However, this view of a very stable genome has started to slowly change as geneticists have become aware of the role plasmids, viruses and transposable elements play in the movement of genetic information, both within the genome of an individual and between species.

In the late 1940's, Barbara McClintock reported a phenomenon in corn that would later be accepted as the first documentation of transposable elements (McClintock, 1956). However, it was not until the 1960's, when transposable elements were found in bacteria, that geneticists really began to accept their existence. Since then, transposable elements have been found in a wide number of organisms and have been shown to be involved in a variety of different hereditary processes. Transposable elements are DNA sequences which can move to new locations in DNA molecules by processes which require neither extensive DNA homology between the element and the site of insertion, nor classical homologous recombination (Berg and Berg, 1983). In most cases, movement results in a new copy of the transposable

element at the site of insertion, while the original copy is retained at the original site. This duplicative transposition results in an increase in copy number of the transposable element within the host's genome.

Although transposable elements have been discovered in a large variety of both prokaryotic and eukaryotic organisms, there is still a tendency to regard them as interesting curiosities in a background of genomic stability. In his review volume entitled "Mobile Genetic Elements", Shapiro (1983) pointed out that one of his objectives was to "show how natural the activity of mobile genetic elements can be in diverse biological situations." He went on to say that even today, "a majority of geneticists would argue that mobile elements are fascinating, but only have significance as part of the background noise of random variability needed to provide the raw material of evolutionary change. We will only integrate mobile elements fully into our picture of heredity when we have formulated entirely new mechanisms for cellular differentiation in both development and evolution."

The behaviour of transposable elements and their significance in evolution has been a controversial issue. On the one hand, their ability to duplicate and transpose has been suggested to be sufficient to ensure their preservation under non-selective conditions or even conditions where there is strong negative selection against the element (Doolittle and Sapienza, 1980; Orgel and Crick, 1980; Hickey, 1982). From this viewpoint,

transposable elements can be considered to be pieces of selfish DNA whose only function is self-replication. On the other hand, it is important to point out the multiple roles that transposable elements can play in a wide variety of organisms which confer a selective advantage on their host.

This thesis deals with the population genetics and spread of one of these transposable elements, the P element of Drosophila melanogaster. From a practical viewpoint, the P element is one of the few eukaryotic transposable elements that can be induced to transpose under the appropriate experimental conditions. More importantly though, the P element presents an interesting problem for population geneticists. First, the only known effect it has, causing hybrid dysgenesis, is detrimental to its host. However, it seems to have spread rapidly through natural populations. The question then arises, how can a transposable element that has a negative effect on its host, increase in frequency in a population? Specifically, the major focus of this thesis is whether P elements can spread through natural populations and if so, under what conditions.

This Chapter reviews what is known about a number of prokaryotic and eukaryotic transposable elements and discusses how these elements may have evolved. Chapters two and three examine several experiments which attempted to model the spread of P elements in natural populations. The experiments described in Chapter four also model the spread of P elements, but they attempt to clarify some of the differences that emerged between

the experimental results outlined in Chapters two and three. Finally, one of the interesting aspects of transposable elements is their ability to regulate their own transposition. Chapter five reviews some of the population genetics models that have been developed to explain the evolution and maintenance of transposable elements in natural populations and discusses how a variety of transposable elements have evolved self-regulated transposition.

Prokaryotic Transposable Elements:

Mobile genetic elements have been found to possess a wide variety of functions in prokaryotes. These elements can mediate the formation of chromosome rearrangements such as deletions, inversions, and duplications of host DNA segments. They can also mediate gene fusion, gene amplification, operon fusion, generation and decay of transposons, replicon fusion and replicon segregation (Shapiro, 1983). Table 1.1 lists some of the transposable elements found in prokaryotes.

IS elements (insertion sequence) were the first transposable elements detected in bacteria. IS elements were initially detected because they induced mutations that had several properties unlike that of point mutations. First, their effects were detectable beyond the border of the mutated gene itself and second, the only form of reversion was deletion of the inserted material (Cohen and Shapiro, 1980). IS elements are arbitrarily defined as a class of prokaryotic transposable elements which

TABLE 1.1: Prokaryotic Transposable Elements.

Element	Length (bp)	Terminal Repeat	Marker	Copy Number*
IS1	768	18/23bp	None	4-10
IS2	1327	32/41bp	None	4-13
IS3	1400	32/38bp	None	5-6
IS4	1426	16/18bp	None	1-2
IS5	1195	15/16bp	None	10-11
Tn3	4957	38bp	Ampicillin	
Tn4	23500		Ampicillin	
Tn5	5400	1500bp	Kanamycin	
Tn7	14000		Streptomycin	
Tn9	2638	18/23bp	Chloramphenicol	
Tn10	9300	1400bp	Tetracycline	
Tn501	7800	38bp	Mercuric Ions	
Tn554	6200		Erythromycin	
Tn903	3100	1050bp	Kanamycin	
Tn951	16600		lac	
Mu	38000		None	

* = On E. coli K12 chromosome

References: Calos and Miller (1980); Iida et al. (1983).

contain no detectable genes unrelated to insertion functions (Campbell et al., 1977). They vary in size from 768 to approximately 5000 bp (basepairs) and normally occur in anywhere from 1 to 13 copies in the chromosome of Escherichia coli K12. They terminate in perfect or near perfect inverted repeats of 15 to 40 bp (Calos and Miller, 1980). Transposition involves (1) local replication of only the IS element and (2) precise DNA cleavage at the ends of the IS element and their ligation to the target DNA (Iida et al., 1983).

Tn elements (transposons) form a second class of prokaryotic transposable elements that contain additional detectable gene(s) unrelated to insertion functions. Transposons (Tn) were originally distinquished from IS elements because they carried detectable genes, which often conferred antibiotic resistence. There are two types of transposons. The Tn3 family of elements have a similar structure and can be thought of as analogous to a single IS element (Heffron, 1983). Each member has a short inverted repeated sequence at either end and they all transpose by a two-step mechanism (Heffron, 1983). Thus the Tn3 family show many differences from composite transposons. Composite transposons are made up of two long repeated sequences at either end (the two IS's) which are able to transpose on their own. Composite transposons generally duplicate a 9 bp sequence of target DNA on insertion whereas Tn3 duplicates a 5 bp sequence. Moreover composite tranposons are not transposed by a discrete two-step mechanism as is Tn3 (Berg and Berg, 1983; Kleckner,

1983). One of the commonly studied composite transposons is Tn5 which encodes resistence to aminoglycoside antibiotics and has the resistence gene bracketed by two copies of IS50. Both insertion sequences IS50L and IS50R can transpose by themselves, however IS50R makes both transposase (the protein necessary for transposition) and an inhibitor of transposition, whereas IS50L makes neither (Berg and Berg, 1983). The IS50R inhibitor of transposition, protein 2, is the smaller and more abundant protein (Johnson et al., 1982) Protein 1 contains an additional 40 amino acids at its N-terminus as compared to protein 2 and has been found to be required for transposition (Johnson and Reznikoff, 1984). Tn10 has a structure similar to Tn5. It is 9.3 kb (kilobase pairs) in length, has inverted repeats of the insertion sequence IS10 at either end, and encodes the gene for tetracycline resistence. IS10R is the functional element and encodes all the necessary functions for transposition, whereas IS10L is defective (Kleckner, 1983). In both Tn5 and Tn10 the left and right IS elements are almost identical. Between IS10L and IS10R there has been about a 2.5% divergence whereas IS50L and IS50R differ by only one bp.

A third class of more complex prokaryotic transposable elements is represented by the bacteriophage Mu; the first prokaryotic transposable element to be described (Taylor, 1963). When Mu infects a host it can enter either the lytic cycle or the lysogenic state. In the lytic cycle all phage functions are expressed and the host cell membrane is lysed, releasing 50-100

phage particles. In the lysogenic state, a repressor is synthesized and blocks the expression of most viral functions and the viral DNA forms a stable association with the host (Toussaint and Resibois, 1983). The difference between Mu and other phages is that the Mu genome integrates into the host chromosome in either state. Moreover, it integrates at a random location, it can transpose to new locations and Mu insertion results in the duplication of 5 bp of the target sequence (Allet, 1979). Therefore Mu is now considered to be a gigantic transposable element 39 kb long (Toussaint and Resibios, 1983).

Eukaryotic Transposable Elements:

Transposable elements have been found in a wide variety of eukaryotic organisms including yeast, Drosophila and plants. Table 1.2 outlines some of the different types of eukaryotic mobile genetic elements and their structure. Four major categories of transposons have now been described in maize: (1) controlling elements, of which approximately eight families have been discovered, (2) a retroviral-like element, (3) Mu, a mutator element discovered in a strain of corn now known as Robertson's mutator and (4) biologically uncharacterized insertions with structures similar to transposons (Lillis and Freeling, 1986). Controlling elements were originally named since insertion of the element at a given site may control the activity of adjacent genes. However, although controlling elements resemble other prokaryotic and eukaryotic elements, they have many striking

TABLE 1.2: Eukaryotic Mobile Genetic Elements.

Organism	Element	Length (bp)	Repeats	Copy Number	Reference
Yeast					
	Ty	5600	330bp	30-35	Roeder and Fink (1983)
	p	330	None	100	—
Corn					
	Ds1	402	11bp	2-¿100	Freeling (1984)
	Ds2	2040	11bp	2-¿100	
	Mu1	1350	235bp		
	Tam1	17000	13bp		
	Cin1	700	14bp		
Vertebrate RNA Retroviruses					
	ASV	3.0-8.6kb	270-350		Varmus (1983)
	REV	5.0-8.0kb	569		
	MLV	4.5-7.8kb	515-588		
	MMTV	6.2kb	1330		

features that are even now unique. They have the ability to sense developmental parameters, transposing and activating other types of genetic change at a predetermined time and with a predetermined frequency (Federoff, 1983; Freeling, 1984).

The Ty elements of the yeast Saccharomyces cerevisiae are a family of dispersed, repetitive DNA sequences occurring 30-35 times per haploid yeast genome (Roeder and Fink, 1983; Fink et al., 1986). Each Ty element consists of a central region of about 5.6 kb of DNA flanked by direct repeats of an approximately 330 bp sequence called δ. The demonstration that Ty elements are transposable came from the analysis of a number of mutations at different loci. Mutations at the HIS4 locus (Roeder et al., 1980) and ADR3 locus (Williamson et al., 1981) have been shown to result from the insertion of Ty elements. One of the more surprising aspects of Ty elements is their heterogeneity. Unlike most other transposable elements, Ty elements have large amounts of sequence divergence in addition to the more common insertion and substitution mutations (Roeder and Fink, 1983). Another interesting aspect of eukaryotic transposable elements is their ability to regulate their own transposition. One way in which Ty element regulation may be regulated is by retention of elements which are tranpositionally non-functional and loss of transpositionally functional elements. It appears that the Ty RNA which is made in in uninduced cells must be largely inactive due to the presence of mutations in the DNA copies from which they came, whereas in transposition-induced cells at least 50% of

the Ty RNA is functional (Lillis and Fink, 1986). There are a number of different types of transposition regulation in both eukaryotic and prokaryotic transposons. These will be discussed in more detail in Chapter 5.

Transposition Mechanisms:

The actual mechanism(s) of transposition have been divided into several categories by different authors (Shapiro, 1979; Bukhari, 1981; Grindley and Reed, 1985). Since this thesis deals with the spread of transposable elements I have chosen to use the categories of Grindley and Reed (1985). Grindley and Reed's (1985) distinction between replicative versus nonreplicative transposition has important evolutionary implications which will be discussed below.

For many transposable elements, transposition involves specific replication of the transposing segment. This is normally acheived by transferring a single strand of the DNA segment; complementary strands are then synthesized in the donor and recipient. This is evidenced by the ability of such elements to form cointegrates. A cointegrate results when two molecules, one of which contains a copy of a transposable element fuse. The cointegrate that results is not simply a fusion of the two molecules, because it now contains two copies of the transposable element. Recombination between the two transposable elements can resolve the cointegrate resulting in two molecules, both having copies of the transposable element (Shapiro, 1979; Bukhari,

1981). Cointegrates are the major product of Mu (during the lytic cycle) and Tn3-like transposons. The second transposition mechanism is a conservative nonreplicative mechanism. In this case, the simplest way to insert an excised unreplicated transposon is a cut and paste mechanism (Grindley and Reed, 1985). Bender and Kleckner (1986) have provided genetic evidence that Tn10 transposes via a non-replicative mechanism.

The evolutionary significance of a replicative versus nonreplicative form of transposition is important. For elements that use a replicative form of transposition (e.g. Tn3), overreplication occurs as a consequence of the transposition process. However, for elements that use a nonreplicative transposition mechanism, overreplication can still occur in several ways. First, the element may spread horizontally on plasmid and phages, and will be amplified as a consequence of overreplication of the transmitting vector. Alternatively, the transposon to chromosome ratio can be increased by loss of the donor chromosome and survival of the single chromosome carrying two copies of the element (Bender and Kleckner, 1986).

Eukaryotic transposable elements can be arbitrarily divided into two groups. The first group, including the I and P elements of Drosophila, do not share any similarities with RNA retroviruses and do not appear to transpose via an RNA intermediate. The mechanism of transposition of the P element will be discussed in more detail later. The second group (eg: yeast Ty elements or Drosophila copia elements) share a number of

properties with integrated proviral retroviruses. These properties include; (1) their sequences are composed of an internal DNA segment several kb long flanked by a pair of identical DNA segments of several hundred bp, which are both arranged in the same direct orientation on the element. These repeated sequences are each bounded by small inverted repeats several bp in length. (2) There is no detectable homology between the DNA sequences at the insertion site and the ends of the element, and (3) the terminal dinucleotides of a transposable element or integrated retroviral provirus are 5'TG...CA3' (Flavell & Ish-Horowicz, 1981; Varmus, 1983; Temin, 1985). These elements probably transpose by RNA intermediates as do the vertebrate retroviruses. Boeke et al. (1985) have shown that Ty transposition occurs through an RNA intermediate. They inserted an intron into a Ty element and then transfected yeast cells. They found that not only did all the Ty mRNA's analysed lack introns, but all the transposed copies of the Ty element that were detected lacked the intron as well. Flavell & Ish-Horowicz (1981) and Shiba & Saigo (1983) have demonstrated that Drosophila copia elements probably transpose through an RNA intermediate.

Transposable elements in Drosophila:

Transposable elements comprise at least one fifth of the Drosophila genome. At least three major classes of transposable element (copia-like, foldback, I and P), distinquishable on the basis of sequence homology, are present as moderately repeated sequences scattered randomly throughout the genome (Rubin, 1983).

Table 1.3 lists the various types of elements and their structures.

Copia-like elements range in size from 5 to 8.5 kb and occur between 10 and 100 times per genome. They are strikingly similar to the yeast Ty elements and to the integrated proviruses of RNA retroviruses. These elements carry long direct terminal repeats at either end, with imperfect inverted repeats at each end of the direct repeat. The terminal repeats of a single element are identical to each other, but they differ substantially between elements in the same family. This would indicate that these elements transpose in a mechanism similiar to retroviruses. Flavell and Ish-Horowicz (1983) found circular extrachromosomal copies of copia elements in cultured Drosophila cells. Some of these copies had both direct repeats, whereas others had only one direct repeat, an observation that is very similar to what is found in retroviral proviruses. Shiba and Saigo (1983) found retrovirus-like particles in cultured Drosophila cells that contained RNA homologous to copia. They suggested that copia-like moveable genetic elements are generally derived from retroviral proviruses which have lost their original properties to various degrees during evolution. However, they point out that it is difficult to distinquish this possibility from the possibility that copia-like elements are themselves the precursors of RNA tumour viruses (Temin, 1980).

The second group of transposable elements found in Drosophila are the foldback or FB elements (Potter et al., 1980;

TABLE 1.3: Transposable Elements in Drosophila.

Element	Length (bp)	Repeat	Copy Number	Insert Duplication
copia	5000	276bp	20-60	5bp
412	7000	481/571bp	40	4bp
297	6500	412bp	30	4bp
mdg1	7200	442bp	20-30	4bp
mdg3	5500	267bp	15	5bp
B104	8500	429bp	100	5bp
gypsy	7300	500bp	10	N.D.
FB1	820	410bp	N.D.	N.D.
FB2	1860	930bp	N.D.	N.D.
FB3	4250	1310bp	N.D.	9bp
FB4	3600	910bp	N.D.	N.D.
FB5	380	190bp	N.D.	N.D.
TE	ç100kb	N.D.	1-2	N.D.
P element	2907	31bp	30-50	8bp
I element	5400	N.D.	30	N.D.

Reference: Rubin (1983).

N.D. = Not done.

Rubin, 1983). These elements range in size from a few hundred bp to several kb in length. Each element carries long terminal inverted repeats and in some cases the entire element consists of these repeats. The inverted repeats are composed primarily of tandem copies of simple sequence DNA. Near the outer ends of each inverted repeat there are multiple copies of a 10 bp sequence; however these 10 bp repeats are generally separated by larger stretches of more diverse DNA.

Hybrid Dysgenesis; P and I elements:

The third group of Drosophila transposable elements are the P and I elements. P and I elements were originally discovered because of their ability to induce a phenomenon called hybrid dysgenesis. Hybrid dysgenesis has been defined as "a syndrome of correlated genetic traits that is spontaneously induced in hybrids between certain mutually interacting strains, usually in one direction only" (Sved, 1976; Kidwell & Kidwell, 1976). These abnormal characteristics include sterility, high mutation rates, male recombination, segregation distortion and chromosome aberrations (Kidwell et al., 1977), and occur when strains carrying either of two independent families of transposable elements, I and P, are crossed with females from the susceptible strains R and M respectively.

The phenomenon of hybrid dysgenesis was discovered in the early 1970's when investigators noticed that hybrids of certain strains displayed a number of dysgenic traits whereas the

reciprocal crosses and parental stocks did not. The actual mechanism of dysgenesis is not known, but it is assumed that P element insertions and excisions, plus chromosome breakage cause the various dysgenic traits (O'Hare, 1985). These traits only occur when crosses are made at temperatures above a certain minimum and they are almost always restricted to germ line tissue. Two independent systems have been identified, the I-R system (Inducer, Reactive) and the P-M system (Paternal, Maternal). Although these systems show striking similarities, they are completely independent, and differ distinctly with respect to DNA structure and sequence (Kidwell, 1979; Bingham et al., 1982; O'Hare and Rubin, 1983; Bucheton et al., 1984).

The characterization of the P-M and I-R systems has been largely achieved using the dysgenic trait of sterility. However, the physiology of this trait differs in the two systems. SF sterility (sterilite femelle) resulting from I-R interactions occurs only in hybrid females; their brothers are normally fertile. SF females lay a normal number of eggs but many of these fail to hatch. Although these eggs are fertilized and initiate meiosis, their development is arrested between the fifth and eight cleavage division (Picard et al., 1977). In contrast to SF sterility, GD (gonadal dysgenesis) sterility resulting from P-M interactions affects both sexes. However, there is a tendency for female sterility frequencies to be somewhat higher than those in males (Engels and Preston, 1979; Kidwell and Novy, 1979). In GD sterility the gonads of both sexes typically do not

develop beyond a rudimentary stage. In addition, a minority of affected hybrids may possess one rudimentary and one normal ovary (Schaefer et al., 1979). A second type of partial sterility has recently been discovered in the P-M system (Kidwell, 1984). EL sterility (embryo lethality) results from embryo death in the progeny of both male and female F1 dysgenic hybrids that have escaped GD sterility at an earlier developmental stage. EL and SF sterility are alike in that both result from death during early F2 embryogenesis, however EL sterility differs from SF sterility in not being restricted to hybrids of the female sex and in showing different patterns of response to temperature and aging in the F1 generation (Kidwell, 1984).

Two factors, temperature and aging influence the development of gonadal traits, particularly sterility. Picard et al. (1977) and Bucheton (1978) found that treatments at high temperature for 48 hours or more during oogenesis of adult females had a curative effect on SF sterility resulting in a sharp increase in hatchability. In contrast, the effect of extended heat treatments earlier in development (larval and pupal stages) consistently reduced the hatching percentage (Bucheton, 1979). GD sterility is highly sensitive to developmental temperatures. Below 24°C, the fertility of dysgenic hybrids is usually not affected, although in one or two exceptional cases GD sterility occurs at 20°C (Bregliano and Kidwell, 1983). Between 24°C and 29°C sterility rises sharply from low levels to a maximum level, with the temperature sensitive stage occurring during late

embryogenesis and early larval development. The effect of age can be seen in the precise timing of embryo death in SF sterility and in the susceptibility of different developmental stages to temperature. In addition to these effects on SF sterility the hatching frequency of eggs increases with the age of the egg-laying hybrid females and eventually reaches control values (Picard et al., 1977). There appears to be no strong effect of aging on GD sterility although aging does have an effect on EL sterility (Kidwell, 1984).

In addition to the inducer and reactive strains in the I-R system and the paternal and maternal strains in the P-M system there are also neutral strains called N and Q in the I-R and P-M systems, respectively. N strains appear to represent one extreme limit of the reactive condition because their level of reactivity is too low to produce any detectable reduction in fertility (Bregliano and Kidwell, 1983). Matings between Q and M strains do not produce GD sterility, however, Q strains mated with M strain females produce nonreciprocal male recombination, lethal mutations and other traits characteristic of dysgenesis (Engels and Preston, 1981; Kidwell, 1981). Q strains are now known to be a subset of P strains which contain sequences homologous to P elements (Bingham et al., 1982), and possess most but not all P strain functions (Bregliano and Kidwell, 1983).

The chromosomal component of the P-M interaction consists of a number of integrated P elements (Bingham et al., 1982). P elements do not appear to occur as extrachromosomal copies

because (1) genetic studies have shown them to be Mendelian factors that can be mapped (Engels, 1979; Kidwell, 1983a) and (2) molecular studies have shown that the elements can be identified by insitu hybridization and cloned elements are all flanked by Drosophila genomic DNA (Bingham et al, 1982; O'Hare and Rubin, 1983). Genetic mapping studies indicate that the potential for GD sterility may be associated with all major chromosome arms of P strains (Engels, 1979; Kidwell, 1983a). Molecular analysis has shown that P elements are dispersed repeated DNA sequences that occur 30-50 times per genome (Rubin et al., 1982). The complete P element is 2907 bp in length with a 31 bp perfect inverted repeat at both ends (O'Hare and Rubin, 1983). In the genome approximately one third of these elements are complete P elements whereas the other two thirds are smaller elements that have arisen from deletions in the larger ancestral element. For the purpose of this thesis I shall use the term P factor to refer specifically to the complete 2.9kb P element which codes for all P functions associated with hybrid dysgenesis, whereas the term P element will refer to all sequences both intact and deleted which are homologous to the ancestral 2.9 kb element (Bregliano and Kidwell, 1983, p. 379). These sequences may, or may not possess all P functions.

Evidence that P elements are transposable and involved in P-M hybrid dysgenesis came from their high mutation rates and spontaneous reversion frequencies (Green, 1977). In addition, Kidwell (1983a) demonstrated a phenomenon called chromosomal

contamination in the P-M system. She was able to show that when M chromosomes which lacked P elements were passed through crosses which combined them with P chromosomes for one or two generations, they gained the ability to induce hybrid dysgenesis. Chromosomal contamination can be most readily interpreted in terms of transposition of P elements from one chromosome to another. Direct evidence of P element transposition was provided by Bingham et al. (1982) and Spradling and Rubin (1982). Using in situ hybridization Bingham et al. (1982) demonstrated that X chromosomes originating from a Canton-S (M) strain were able to acquire on average 0.8 P element copies per fly per generation. Spradling and Rubin (1982) microinjected plasmid DNA containing a cloned copy of the 2.9 kb P element into M-type embryos. They demonstrated that transposition of the P element into M-type chromosomes can occur.

Although the actual mechanism of P element transposition is not known, there are a number of details that are understood. P elements do not appear to transpose via an RNA intermediate. This prediction is supported by several lines of evidence. First, P elements have the terminal dinucleotides 5'CA...TG3' in contrast to the terminal dinucleotides 5'TG...CA3' of transposable elements that transpose via an RNA intermediate (Varmus, 1983; Temin, 1985; p. 13). Second, they lack the long terminal repeats characteristic of retroviral proviruses and finally, the major P element transcripts lack the first 87 nucleotides, making them unlikely transposition intermediates

(O'Hare and Rubin, 1983; O'Hare, 1985). The best picture of P element transposition at the moment is that of a cut and paste mechanism where the transposase introduces double-stranded cuts in the target DNA and at the ends of the element. The sequences of the element are then copied into or physically moved into the new site, depending on whether transposition is replicative or not. Physical movement of the P element into a new site without replicative transposition would be similar to what has been found for Tn10 (Bender and Kleckner, 1986). In contrast, replicative transposition would presumably occur similar to Tn3 which transposes through the formation of a cointegrate. Deletions could result from slippage of the replication fork during replicative transposition (O'Hare, 1985).

Perhaps the most basic question pertaining to the spread of P elements is to resolve whether transposition is a replicative process or not. To date this question has been difficult to resolve. Transposition only occurs in the germline, and it is impossible to guarantee recovery of all products of meiosis in D. melanogaster. Thus the observation that two copies of an element are present in the progeny of a fly where the parent has only one copy does not establish that transposition is replicative. It remains possible that the second copy arose by non-replicative transposition from a sister chromatid after DNA replication. However, there are several lines of evidence that suggest that both P and I elements transpose replicatively. For I elements it has been shown that a chromosome retains its inducer properties

after it has acted as a contaminating element (Picard, 1976). Second, the mutation frequency of the w^{IR1} element is at least 100 times lower than that of the contaminating frequency closely linked to this allele (Pelisson, 1981). This implies that most of the time transposition does not change the w^{IR1} DNA sequence. The evidence that P elements transpose replicatively is rather weak. Kidwell (1983) demonstrated that certain chromosomes can act as contaminating elements, but she did not test whether these chromosomes retain their P element activity after contamination. Rubin (Unpublished) has found in several cases that after flies had been transformed with P elements, the number of P elements in the offspring of these flies would increase in subsequent generations.

P elements are mobilized in a maternal cytoplasmic state called M cytotype which confers on the genome a susceptibility to the action of these elements. The common view is that cytotype is determined by the P element itself which codes for its own transposase and regulator (O'Hare & Rubin, 1983; Karess & Rubin, 1984). In P lines, the regulator prevents transposition, whereas when a P male is crossed to an M female, the lack of regulator in the M egg permits the production of transposase and allows transposition to occur. The pattern of inheritance suggests that cytotype is maternally inherited and can be maintained over one or a few generations, but its long term maintenance is based on chromosomal factors, presumably P elements (Engels, 1979; Kidwell, 1981). The slow shift from M to P cytotype over several

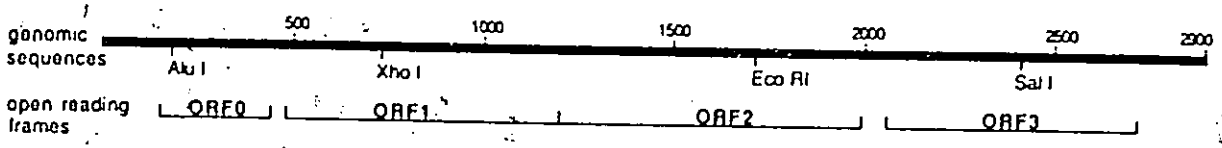
generations, in the presence of P elements has been difficult to explain. Since any non-replicating factor would be subject to enormous dilution, Engels (1979, 1981) suggested that cytotype is determined by an episome with a limited potential for self-replication. However, there are several lines of evidence that suggest that P element regulation is chromosomally inherited. First, Kidwell (1985) has shown that P element regulation can be mapped to different chromosomes, indicating a Mendelian component in regulation. Second, since removal of the intron between ORF2 and ORF3 of the P factor results in a P element that does not have tissue specific regulation, one would assume that chromosomally integrated P factors are capable of P element regulation. O'Hare and Rubin (1983) suggested that P factors code for at least two functions, a transposase and a regulator that can lead to the suppression of transposition. If the regulator positively feeds back to stimulate its own activity, then this could explain the self-reproduction of cytotype. For example, in a dysgenic cross, P element DNA is introduced into a background where there is neither transposase nor regulator. Expression of both functions leads initially to transposition, however with time, positive feedback by the regulator on its own level of activity produces enough regulator to shut off transposition and the P cytotype is established.

The 2.9 kb P factor contains 4 open reading frames (ORF's) (Figure 1.1). Deletions in any of these ORF's abolishes the ability of the cloned element to transpose (Karess and Rubin,

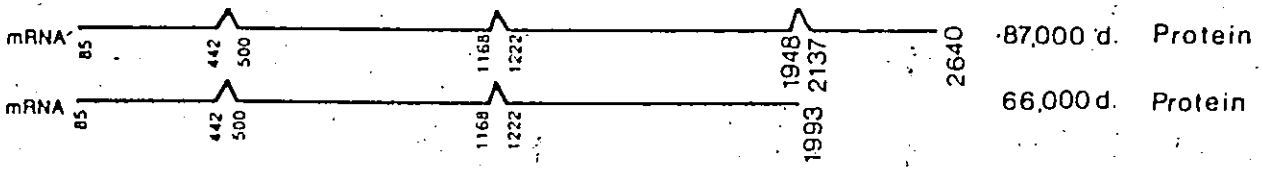
FIGURE 1.1: Schematic diagram of the P factor. The P factor is 2907 bp in length, with four open reading frames (O'Hare and Rubin, 1983; Karess and Rubin, 1984). The two transcripts represent the DNA sequences which encode the 87,000 dalton protein (the transposase) and the 66,000 dalton protein (the putative regulator) respectively (Rio et al., 1986). The Alu1, Xho1, Eco R1 and Sal1 restriction enzyme sites show the locations of the frameshift mutations constructed by Karess and Rubin (1984) that demonstrated that all four ORFs are required for the production of P element transposase.

The P Element

STRUCTURE



PROTEIN PRODUCTS



1984). P element transposition is now known to be caused by an 87,000 dalton protein called a transposase (Rio et al., 1986). This protein has been shown to be required for at least one of the biological activities associated with P element induced hybrid dysgenesis, namely precise and imprecise P element excision (Engels, 1983; Rio et al., 1986). The tissue specificity of P element transposition has been shown to result from differential splicing of the RNA transcript (Laski et al., 1986). In germ line cells the intron between the second and third open reading frame is spliced out, making an active transposase, whereas in somatic cells this splicing does not occur. The tissue specific mechanism of splicing is not known, but P elements lacking the intron between the 2nd and 3rd open reading frame produce a transposase that is active in somatic cells (Laski et al., 1986). Rio et al. (1986) also demonstrated that the P factor produces a 66,000 dalton protein which they suggest might function to negatively regulate P element transposition. They argue that the 66,000 dalton protein might negatively regulate transposition by either binding in a competitive manner to the P element termini, or by a direct protein-protein interaction with the 87,000 dalton transposase protein.

The molecular basis of I-R hybrid dysgenesis is a bit more complex than that in the P-M system, but has not been nearly as well studied. The I element appears to be a 5.4 kb sequence, however, all strains of D. melanogaster that have been analysed

(including both I and R strains) contain copies of the I element (Bucheton et al., 1984). Bucheton et al. (1984) felt that the I sequences in reactive strains cannot represent active I elements. They demonstrated that I elements have a remarkably similar sequence organization in all R strains and are located in pericentromeric regions of the chromosomes. Inducer strains appear to contain both incomplete I elements located in the pericentromeric regions and 10-15 copies of the complete I element at sites on the chromosome arms.

Natural History and Distribution of P and I Elements:

At the population level, the frequency of P and I strains has been found to be correlated with the laboratory age of the stock. In the P-M system there were no P strains collected in the wild until 1950, but since then the frequency of P strains has increased rapidly (Table 1.4; Kidwell, 1983b). Recent wild collections are almost entirely P-type although a few M wild populations still do exist (Bregliano and Kidwell, 1983; Anxolabehere et al., 1984). A similar situation occurs in the I-R system. Of the samples collected in the 1920's, very few were I strains whereas wild flies caught in the 1970's all contained I elements. Two hypotheses have been proposed to account for this relationship between the strain distribution of P and I elements and the laboratory age of the stock. The "stochastic loss hypothesis" suggests that P elements have always been present in substantial frequencies in natural populations, and that the

TABLE 1.4: Distribution of *Drosophila melanogaster* strains collected from nature according to both systems of hybrid dysgenesis.

System	Decade of collection in the wild					
	1920's	1930's	1940's	1950's	1960's	1970's
P-M system						
Americas						
M	100%	100%	-	87%	58%	3%
P or Q	0%	0%	-	13%	42%	97%
(n)	3	8		15	38	304
Other Cont.						
M	100%	100%	100%	92%	97%	32%
P or Q	0%	0%	0%	8%	3%	68%
(n)	1	5	4	24	32	34
I-R system						
Americas						
R or N	67%	50%	-	13%	3%	0%
I	33%	50%	-	87%	97%	100%
(n)	3	4		15	38	304
Other Cont.						
R or N	100%	100%	75%	27%	9%	0%
I	0%	0%	25%	73%	91%	100%
(n)	1	5	4	26	33	34

n = Total number of strains analysed

Reference: Bregliano and Kidwell (1983).

apparent distribution of strains over the last 50 years is the result of loss of elements from laboratory populations, possibly by random genetic drift in small populations (Engels, 1981; Kidwell, 1983b). The second hypothesis, the "recent invasion hypothesis" proposes that until recently P elements were absent from natural populations or occurred at very low frequencies. The P elements then spread rapidly through replicative transposition, with or without positive or negative selection (Kidwell et al., 1981; Kidwell, 1983b).

Kidwell et al. (1981) and Kiyasu and Kidwell (1984) have studied the ability of P elements to survive and spread in mixed P-M populations. Kidwell et al. (1981) found that at 20°C, a condition where there was no negative selection against P elements, mixed P-M populations change unidirectionally towards the P-type fairly rapidly. Under conditions of strong negative sterility selection (27°C), Kiyasu and Kidwell (1984) found that most mixed populations evolved to the P-type, however the equilibrium level of P element activity was lower than in the earlier experiments at 20°C (Kidwell et al., 1981). This suggests that selection favours P elements with weak rather than strong sterility potential. Kidwell (1986) introduced P elements into M strain genomes, and found that 4 of the 8 experiments rapidly increased their potential for P element activity to close to the 100% maximum. She also confirmed the presence of multiple P elements in each line by restriction enzyme analysis. The conclusion from all these studies was, that due to replicative

transposition, P elements have spread rapidly through these populations.

Evolution of Transposable Elements:

The evolution of transposable elements can be best dealt with by separating it into two questions. First, where did mobile genetic elements originally come from? Second, what forces account for their maintenance and spread in natural populations?

Hartl et al. (1984) argued that the subtle favourable effects of bacterial transposons observed in chemostats (See below), represent individual selection of the sort that favoured the establishment and proliferation of such DNA elements in the first place. Their over-replication and horizontal transmission then became established and the elements took on dynamics of their own. At the same time there was the original impetus of population-level selection which further modified and refined these elements. However, there is no reason why a transposable element should not have first evolved the ability to over-replicate and transmit horizontally and then evolve the more subtle effects found in chemostats.

In eukaryotic organisms, the similarity between transposable elements and retroviruses has lead many people to speculate that one evolved from the other (Temin, 1980, 1985; Varmus, 1983). Temin (1980) argued that retroviruses have evolved from transposable elements. Basically, he suggests that retroviruses

may have evolved much in the same fashion that prokaryotic transposons did. Two small insertion sequences could transpose around the genes necessary for the virus to move through the process of transcription, reverse transcription and integration. On the other hand, Shiba and Saigo (1983) argue that the copia-like transposable elements evolved from retroviruses. However, there is very little evidence to support or refute either of these suggestions. At the moment it is impossible to say whether copia-like transposable elements are evolving towards retroviruses or descending from them. The transposable elements that do not resemble retroviruses may have a completely different evolutionary history or they may have diverged sufficiently from their viral ancestors so that there is no longer any structural resemblance between them.

My personal opinion is that eukaryotic transposable elements are more likely the precursors of retroviruses than vice-versa, for two reasons. First, if one argues that retroviruses are the precursors to transposable elements, that simply creates another question; how did retroviruses evolve into the complex structure that they currently display. Second, retroviruses are found only in vertebrates, therefore from a phylogenetic point of view it seems easier to accept that transposable elements were the precursors to retroviruses than vice versa.

What forces have acted in the maintenance and spread of transposable elements? There are two competing hypotheses to explain the evolution of transposable elements. The first

hypothesis is that mobile genetic elements are sustained by direct selection. They offer some distinct selective advantage to their host and have spread because they increase the fitness of their host. The second hypothesis is that transposable elements do not need to confer any selective advantage on their host, but can spread as selfish DNA as a result of their ability to undergo replicative transposition.

The Selective Advantage Hypothesis:

Transposable elements can confer a selective advantage on their host either directly as in the case of antibiotic resistence, or indirectly, as selection acting on genetic variability.

The prokaryotic transposable elements which confer drug resistence on their host all appear to have spread rapidly as a result of the widespread usage of antibiotics after World War II. The emergence of antibiotic resistance involved the introduction of a novel antibacterial agent followed by the rapid appearance of resistent bacterial populations which became more and more widespread, both geographically and also with respect to the bacterial strains and species exhibiting resistence (Mitsuhashi, 1971; Richmond, 1983; Datta, 1984). Hughes and Datta (1983) used a collection of strains isolated from 1917 to 1954 and demonstrated that the medically important bacteria have acquired antibiotic resistence in the past 25 years by insertion of new genes into existing plasmids rather than by the spread of previously rare plasmids.

It has also been suggested that transposable elements can provide a selective advantage to their host by inducing mutations which increase the level of genetic variability in the host (Syvanen, 1984). This argument is similar to one originally proposed for mutator genes in bacteria. It has been known for some time that populations harbouring mutator genes that increase mutation rates in Escherichia coli are at a strong selective advantage in competition experiments (Cox, 1976; Chao and Cox, 1983). For example Chao and Cox (1983) found that when E. coli carrying the mutT gene (which increases mutation rates 100 to 1000 times) were placed in a chemostat with wild type cells, no difference in growth was observed in the first 50-150 generations, after which the mutT containing strain increased its relative proportion by about 200 fold over the next 50 generations. They demonstrated that mutator bacteria acquired the advantage by evolving faster. This advantage is frequency dependant. The mutator strain is favoured only above a starting ratio of approxiamtely $5x10^{-5}$, and results from the fact that the necessary beneficial mutations cannot be generated in a population below a certain size. Chao et al. (1983) considered the possibility that transposable elements confer an advantage in the same manner as mutator genes. They found that Tn10 also confers an advantage in chemostats, and that this advantage is frequency dependent. When the Tn10 containing strain predominated, a segment of Tn10, probably the IS10 sequence was found to have undergone transposition into a new site, whereas

Tn10 from the chemostats where the wild type strain won, did not show additional copies of Tn10. In the four separate experiments where Tn10 won, the new IS10 element was found to be inserted in a PvuII restriction fragment of 3.2 kb, suggesting that the same mutational event is selected each time. Biel and Hartl (1983) and Hartl et al. (1983) looked at the same problem by performing chemostat competition experiments with bacteria containing IS50R, but observed very different results. They found that the IS50R strains grew more rapidly for about 50 generations, and that the competitive advantage was immediate upon inoculation and showed no frequency dependence. This indicates a direct growth advantage for the strain containing IS50R. They also found that when the victorious IS50R clones were analysed, there was no evidence of IS50R mediated rearrangements or transpositions. The most reasonable explanation for these events is that IS50R confers a direct growth advantage to its host. The selective advantage of a transposable element in inducing mutations will be similar to Painter's (1975) model for optimizing mutation rates in asexual populations. Consider various populations of bacteria, some with transposable elements which cause mutations, in a habitat that fluctuates every fixed number of generations. With every fluctuation the population carrying the transposable element will respond with an adaptation and rise to a new plateau. The strains containing transposable elements will increase the most in frequency, because they are much more likely to produce a favourable mutation. Those same strains will

however also generate more deleterious mutations and will in turn decrease in numbers at a faster rate at the new plateau, until the onset of new fluctuations. The end result will be a transposition rate that maximizes the difference between the gain during the rise phase and the loss during the plateau (Chao and Cox, 1983). Thus prokaryotic transposable elements may be capable of spreading and maintaining themselves in natural populations by inducing mutations that are positively selected for.

In eukaryotic organisms, the argument has also been made that transposable elements can be selected for because of their ability to create new genetic variation. It was originally thought that the spread of P and I elements might have resulted from the widespread use of pesticides in the last 40-50 years (Bregliano and Kidwell, 1983). For example, if the transposition of P and I elements caused mutations that resulted in more resistant individuals within a population, they would be highly selected for. The only study which has demonstrated that a eukaryotic transposable element can increase the genetic variation in a population is that of MacKay (1984; 1985). MacKay used abdominal bristle number, a polygenic trait that has often been used for selection experiments in the past, to see if P element transposition accelerated the response to selection for bristle number. She found a significant amount of the new mutational variation affecting bristle number was generated by the activity of transposable elements mobilized during hybrid

dysgenesis. The phenotypic variance of abdominal bristle score increased by a factor of four in lines selected for dysgenesis in comparison with nondysgenic lines. Much of this new variation was deleterious judging by the overall reduction in viability of chromosomes extracted from selected lines.

The Selfish DNA Hypothesis:

The competing, selfish DNA hypothesis asserts that the maintenance and distribution of DNA sequences such as transposable elements can be understood in terms of the selective advantage to the elements themselves as a result of their ability to multiply within a genome, and not to any advantage they may provide to the individuals who carry them (Doolittle and Sapienza, 1980; Orgel and Crick, 1980). These elements could have a negative effect on the fitness of their host and still spread through the popultion as a result of replicative transposition (Hickey, 1982). There are a number of eukaryotic transposable elements that have been found to have a negative effect on the fitness of thier host. For example transposition of the yeast Ty elements has been found to cause mutations in the HIS4 and ADR3 locus (Roeder and Fink, 1983) and the ADH2 and CYC7 locus (Eibel and Philippsen, 1984). However, the classic example of transposable elements reducing the fitness of their host remains the case of P and I elements. The argument is often made that although some transposable elements may appear to lower the fitness of their host under certain conditions, their existence must result from them providing some selective advantage to their

host, and we have simply not been able to discover what this advantage is. This is an argument that cannot be easily countered, and in my opinion offers little value in the discussion of the evolution of selfish DNA.

There are clearly a wide variety of different transposable elements in nature, some of which confer a direct selective advantage to their host (eg: Tn5, Tn10, etc). Others appear to have little or no effect on the fitness of their host, whereas a third group clearly has a detrimental effect on host fitness. A number of different models have been developed to explain the evolutionary dynamics of transposable elements (Hickey, 1982; Langley et al. 1983; Ohta, 1983; Charlesworth and Charlesworth, 1983; Charlesworth and Langley, 1986). Chapter 5 will consider in more detail the evolution of transposable elements and discuss some of the population genetics models that have been developed.

The Spread of P elements:

This thesis was undertaken in order to understand how P elements can, on the one hand, reduce the fitness of their host, and on the other hand appear to spread very rapidly through natural populations. One model to explain the spread of P elements, argues that P elements have spread rapidly as a result of replicative transposition. However, there are several possible mechanisms to explain the evolution of a P-type population which do not require any replicative transposition. As previously discussed, P elements could code for a product that

in some way increases the fitness of their host, analogous to the antibiotic resistence encoded by many bacterial transposons. Second, P strain chromosomes could possess an intrinsic fitness advantage over M chromosomes unrelated to the presence of P elements. Finally, the number of P-type flies could increase in a population because of chromosomal segregation and recombination. This third possibility is particularly important because none of the studies to date have distinquished between the spread of P-type flies through chromosomal segregation and recombination versus spread as a result of P element transposition.

P elements occur 30-50 times per genome and seem to occur on all major chromosome arms (Bingham et al. 1982; O'Hare 1985). Through segregation, the frequency of flies carrying P elements would be expected to increase in each generation, although the number of elements per fly is actually decreasing. For example, if one were to start with 10% P flies in a population, each having 50 copies of the P element after two generations of segregation approximately 34% of the flies would have on average 14.5 P elements per genome. Table 1.5 illustrates how there can be an increase in the number of P-type flies or P genotypes (those flies containing one or more copies of the P element), in a population without an increase in the total number of P elements through replicative transposition. Figure 1.2 provides a diagram of this phenomena. As the P elements segregate throughout the population, there is an increase in the number of

flies carrying P elements, but a corresponding decrease in the number of P element copies per fly. One question that would then arise is how does this reduced number of P elements within an individual fly affect its ability to induce hybrid dysgenesis.

To date, it has not been convincingly demonstrated that P elements can spread through a population as a result of replicative transposition. In order to clearly demonstrate this it is necessary to show (1) an increase in the number of P-type flies and (2) an increase in the number of copies of the P element in the population. The experiments discussed in this thesis were designed to model the introduction of a small number of P-type flies into a large M-type population. By being able to look at the P element activity (as measured by the traditional method of gonadal dysgenesis) and copy number of the P element my aim was to clearly understand how P elements spread through natural populations.

TABLE 1.5: Theoretical changes in the frequency of P-type flies (P genotypes), and P element copy number in a randomly mating population, assuming no replicative transposition or selection. These examples are (a); for a mixed population containing 1% P-type flies and 99% M-type flies at generation 0 and (b); for initial frequencies of 10% P-type and 90% M-type flies.

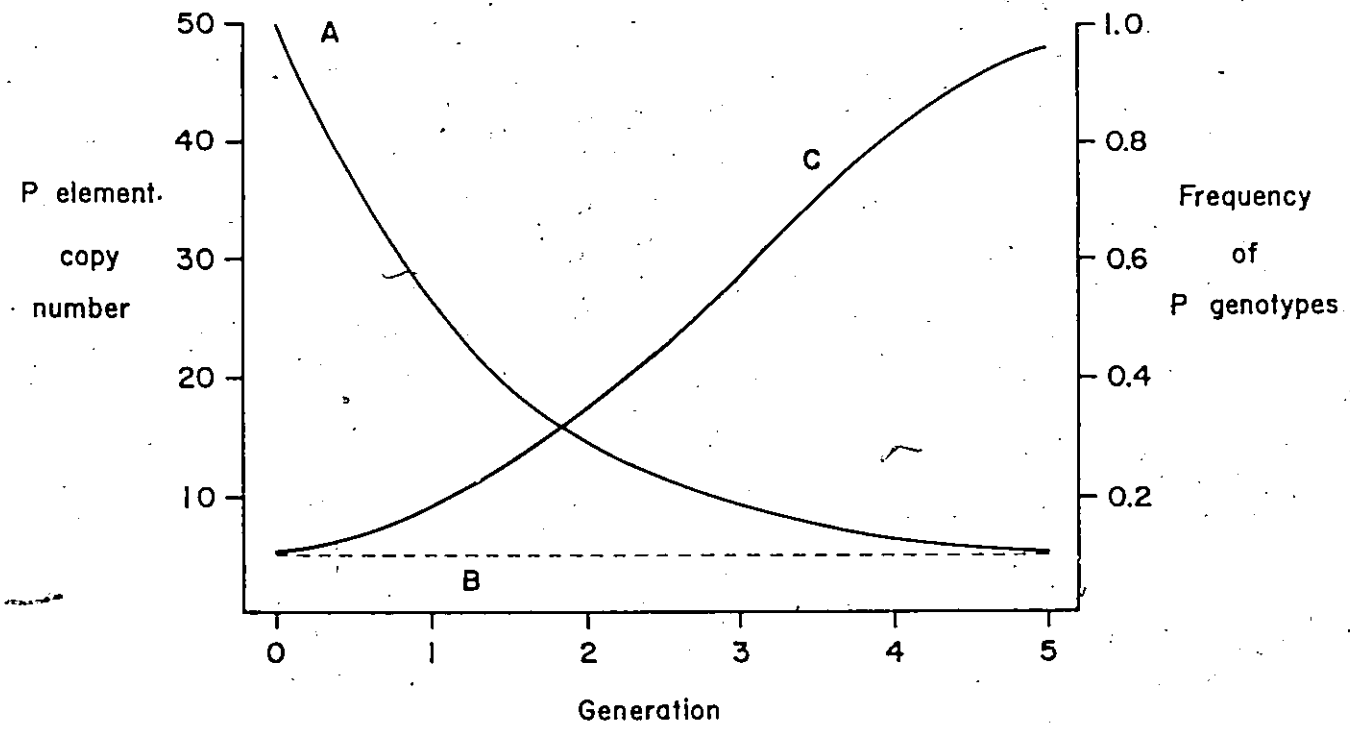
Generation	% P-type flies (A)	P element copy # in P flies (B)	Average copy # per fly (AxB)
(a)			
0	1%	50	0.5
1	2.0%	25.1	0.5
2	3.9%	12.7	0.5
3	7.7%	6.47	0.5
4	14.9%	3.36	0.5
(b)			
0	10%	50	5
1	19.0%	26.3	5
2	34.4%	14.5	5
3	57.0%	8.77	5
4	81.5%	6.13	5

FIGURE 1.2: Theoretical changes in the frequency of P-type flies (P genotypes) and P element copy number in a randomly mating population, assuming no replicative transposition or selection. The initial frequency is 10% P-type flies and 90% M-type flies. See Table 1.5 for detailed calculations.

A = P element copy number in P-type flies

B = Average P element copy number per fly

C = Percentage P-type flies


50
40
30
20
10
P element copy number
A
B
C
0
1
2
3
4
5
Generation
1.0
0.8
0.6
0.4
0.2
Frequency of P genotypes

CHAPTER 2

THE LOSS OF P ELEMENTS THROUGH REPEATED BACKCROSSING TO AN M-TYPE STRAIN

Introduction:

One of the interesting aspects of P elements is their distribution in nature. As stated in the previous chapter, it has been proposed that until recently P elements were absent from natural populations or occurred at very low frequencies. P elements then spread rapidly through replicative transposition, with or without positive or negative selection (Kidwell et al., 1981; Kidwell, 1983b).

There are several possible mechanisms to explain the evolution of a P-type population which do not require any replicative transposition. First, P elements could spread because they confer on their host a selective advantage, analogous to antibiotic resistence encoded by bacterial transposons. Second, P strain chromosomes could possess an intrinsic fitness advantage over M chromosomes unrelated to the presence of P elements. Finally, the number of P flies could increase in a population because of chromosomal segregation and recombination. Through segregation, the frequency of flies carrying P elements can increase in each generation, although the number of elements per fly is actually decreasing (Chapter 1).

The rationale for the experiments described in this chapter is as follows. Suppose one or a few migrant P flies were to invade a large M-type population. During the initial stages of P

element invasion these migrants and their offspring would be most likely to mate with indigenous M-type flies. Only after the frequency of P elements had increased to a reasonable level would P X P type mating become common. One way to model this situation would be to repeatedly backcross P flies to M flies. These backcrosses would dilute the P element 50% each generation and if done at high temperatures selection against dysgenic hybrids could result in even stronger selection. One prediction would be that replicative transposition of the elements should counteract, at least in part, the dilution effect of the backcrosses.

Since the spread of P-type flies in a natural population does not necessarily indicate that P elements are undergoing replicative transposition it must be possible to distinquish between (1) an increase in the number of P-type flies in a population and (2) an increase in the number of copies of P elements in a population, in order to properly answer this question. The number of P-type flies was measured by the usual method of gonadal dysgenesis (Engels, 1979; Schaefer et al., 1979) whereas the average copy number of P elements was measured by DNA dot hybridization using a P element molecular probe. Specifically, these experiments test whether P elements can spread under these conditions of strong negative selection and, if so, how quickly. Second, can DNA hybridization provide an accurate measure of the number of P elements within a fly population.

Materials and Methods

Drosophila Stocks:

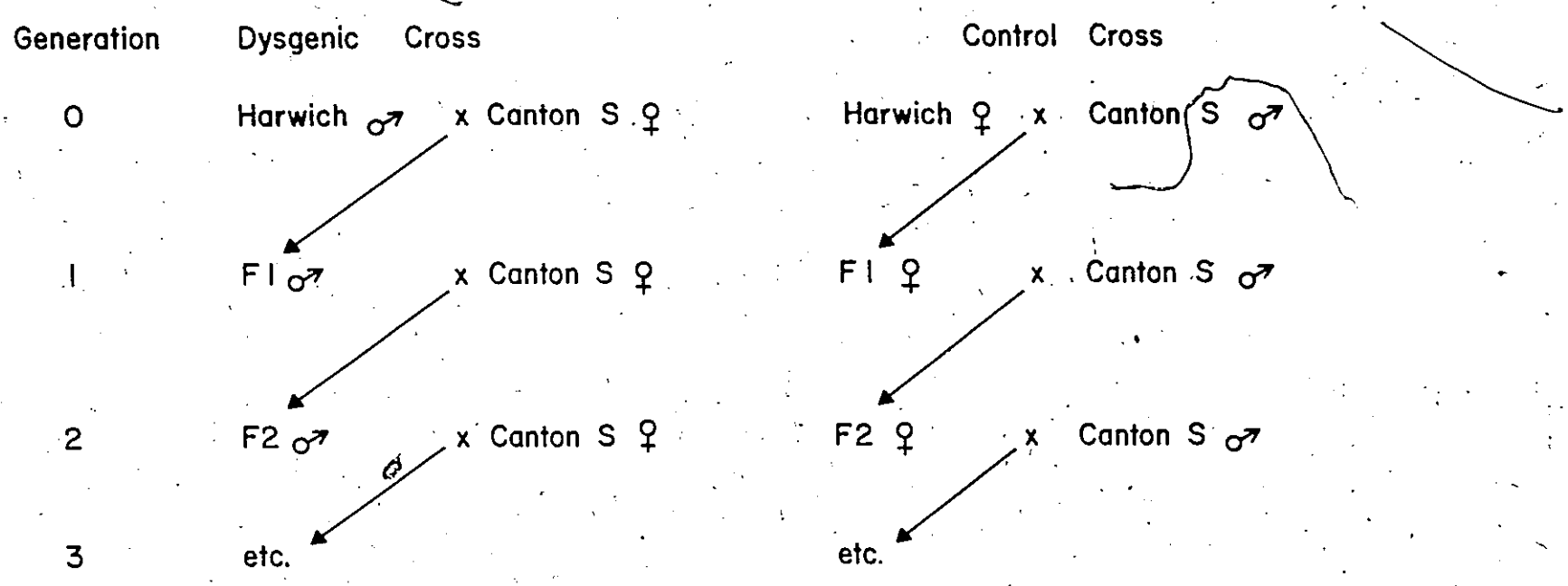
1. Harwich: An inbred wild-type P strain collected from the wild in 1967 by M.L. Tracey, Jr. A white eyed mutant of this stock was isolated by M. Kidwell and used as one of the P stocks and as the standard reference P strain to test cytotype (Kidwell et al., 1981).

2. Canton-S: A standard wild-type laboratory M stock that was used as the M stock and to test for P element activity.

All cultures were grown on a Carolina Instant Drosophila medium and maintained at 21°C except where otherwise stated.

Scheme of backcrosses: The mating scheme used in the backcrosses is shown in Figure 2.1. All matings were made en masse with at least 30 parents of each sex and the flies raised on Carolina Instant Drosophila medium in standard shell vials (95mm Ht x 25 mm Dm). Three replicate populations were set up for each experiment. In the dysgenic cross (A), Harwich males were mated to Canton-S females and for each generation the F1 males were backcrossed to Canton-S females. Since these crosses are potentially dysgenic, P elements should transpose in this cross and be able to maintain a high copy number. In the control cross (B), Harwich females were mated to Canton-S males and each generation the F1 females were backcrossed to Canton-S males. In these nondysgenic crosses no transposition should occur, at least in the initial generations because the F1 females should be P cytotype. Each generation, the males were tested for P element

FIGURE 2.1: Mating scheme used in the backcross experiments. In the dysgenic cross (A), Harwich (P) males were mated to Canton-S (M) females. In the control cross (B), Harwich (P) females were mated to Canton-S (M) males.


Generation
Dysgenic Cross
Control Cross
0
Harwich ♂ x Canton S ♀
Harwich ♀ x Canton S ♂
1
F1 ♂ x Canton S ♀
F1 ♀ x Canton S ♂
2
F2 ♂ x Canton S ♀
F2 ♀ x Canton S ♂
3
etc.
etc.

activity. In generations three and six flies were frozen and their DNA extracted as outlined below. These experiments were performed at three temperatures, 27°C, 24°C and 21°C. At 27°C there should be a high level of transposition as indicated by the high rates of dysgenesis (Engels, 1979) whereas at 21°C transposition does occur, (Bingham et al., 1982) but no dysgenesis occurs.

Method of P element activity determination: Individual males were mated with 3-4 Canton-S females, the F1 hybrid females were aged 2-4 days and their ovaries dissected according to the methods of Schaefer et al., (1979). Ten females from each individual mating were scored for sterility and the percentage dysgenesis was calculated as the mean of 10 individual matings.

Statistical Analysis: All statistical analyses were performed using the SAS statistical package programs on the University of Ottawa's Amdhal V8 mainframe computer. Analysis of variance and Duncan's multiple range tests were performed using the PROC GLM statement of SAS.

Detection of P elements in crosses by hybridization: The presence of P element sequences in a particular generation was investigated by DNA hybridization. DNA was extracted from 100-500 flies by the method of Strausberg and Keifer (1979). Genomic dot blots were performed using a Schleicher and Schuell Minifold on Biodyne nylon membrane following the procedure of Kafatos et al. (1979). The DNA was bound to the filter by UV crosslinking using the technique of Church and Gilbert (1984). Two 32-P

labelled probes were used: as a single copy gene, the 7.2 kb HindIII fragment of the C20 plasmid which contains the genomic sequences from the Xanthine dehydrogenase gene of Drosophila melanogaster (Figure 2.2; Rubin and Spradling, 1983), and as a P element probe, the 0.84 kb Hind III (HIII) fragment from the p π25.1 plasmid (Figure 2.2; Spradling and Rubin, 1982). The HIII fragment is located on the left hand side of the P element and spans from 39 bp to 877 bp of the complete P element. The choice of the HIII fragment as a P element probe was due to several factors. Preliminary experiments demonstrated that using the entire pπ25.1 plasmid had several problems. First there were high levels of background which made copy number calculations difficult. In addition, pπ25.1 contains white sequences from Drosophila which made the analysis much more difficult. Finally, since many P elements have internal deletions, an internal restriction fragment would not bind to some deleted elements. This fragment was chosen since it should bind to all P elements, both deleted and complete (O'Hare & Rubin, 1983). The choice of the HIII has the disadvantage that it does not permit the distinction between P factors and P elements, this problem will be discussed in detail later.

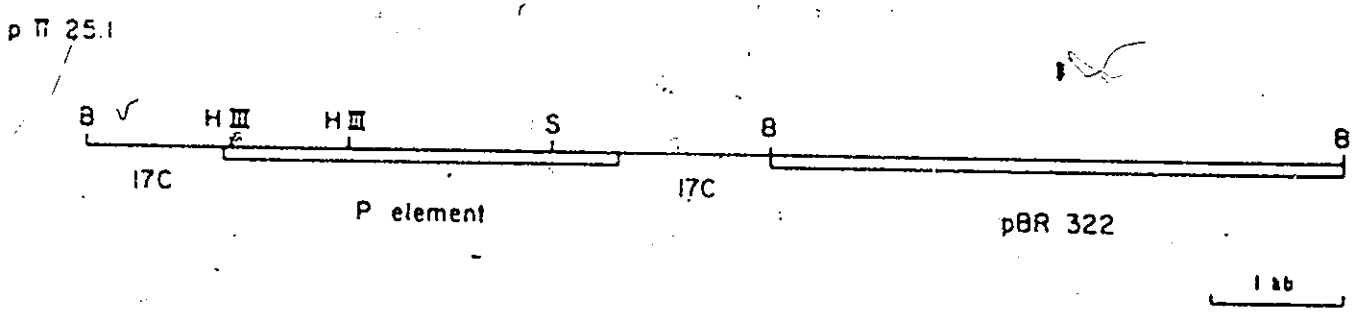
Both fragments were purified twice, by cutting the appropriate restriction fragment from an agarose gel and eluting the DNA with an ISCO 1750 sample concentrator. The fragments were nick translated to a specific activity of approximately $1x10^8$ cpm using the protocol of Maniatis et al. (1982; p.109).

FIGURE 2.2: Schematic diagram of the two plasmids pπ25.1 and C20. pπ25.1 contains the complete P element with some 17C Drosophila melanogaster flanking sequences, cloned into the Bam HI (B) site of pBR322 (Rubin and Spradling, 1982). C20 contains the genomic sequences coding for Xanthine dehydrogenase which has been inserted into a deleted P element. The P element is flanked by Drosophila white sequences and cloned into pUC8 (Rubin and Spradling, 1983). The restriction enzymes used are:

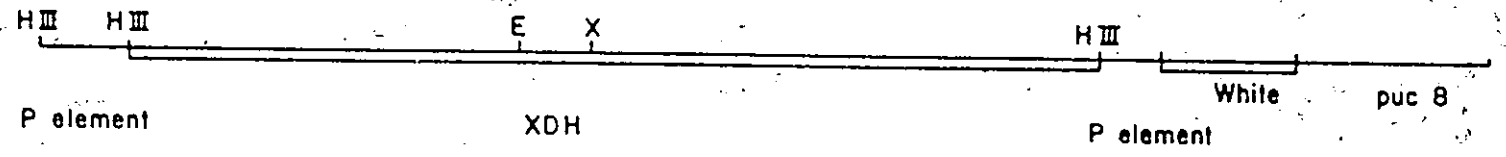
B = Bam HI E = Eco RI

HIII = Hind III S = Sal I

X = Xho I


p π 25.1
B
H III
H III
S
B
B
17C
17C
P element
pBR 322
1 kb

C20


H III
H III
E
X
H III
White
puc 8
P element
XDH
P element

Prehybridization and hybridization were carried out at 65°C without formamide using the protocol of Maniatis et al. (1982; p.387) and the filter was then washed three times for thirty minutes each in 5mM Sodium Phosphate pH 7.0, 1mM EDTA, 0.2% SDS.

The percentage of P elements in a cross in comparison to Harwich was calculated as follows. Dot blots were probed with XDH, washed in 10mM sodium phosphate, 50% formamide at 65°C, then reprobed with the P element probe. Each autoradiogram was then scanned using a Vitatron TDL 100 densitometer. Based on a dilution series the relationship between DNA concentration and densitometer reading was found to be linear unless the dots being scanned had saturated the X-ray film. Using three replicate dots, the ratio of P element signal to XDH signal was calculated for the Harwich strain and each of the crosses. The ratio of P elements in each cross compared to Harwich was then calculated using the formula:

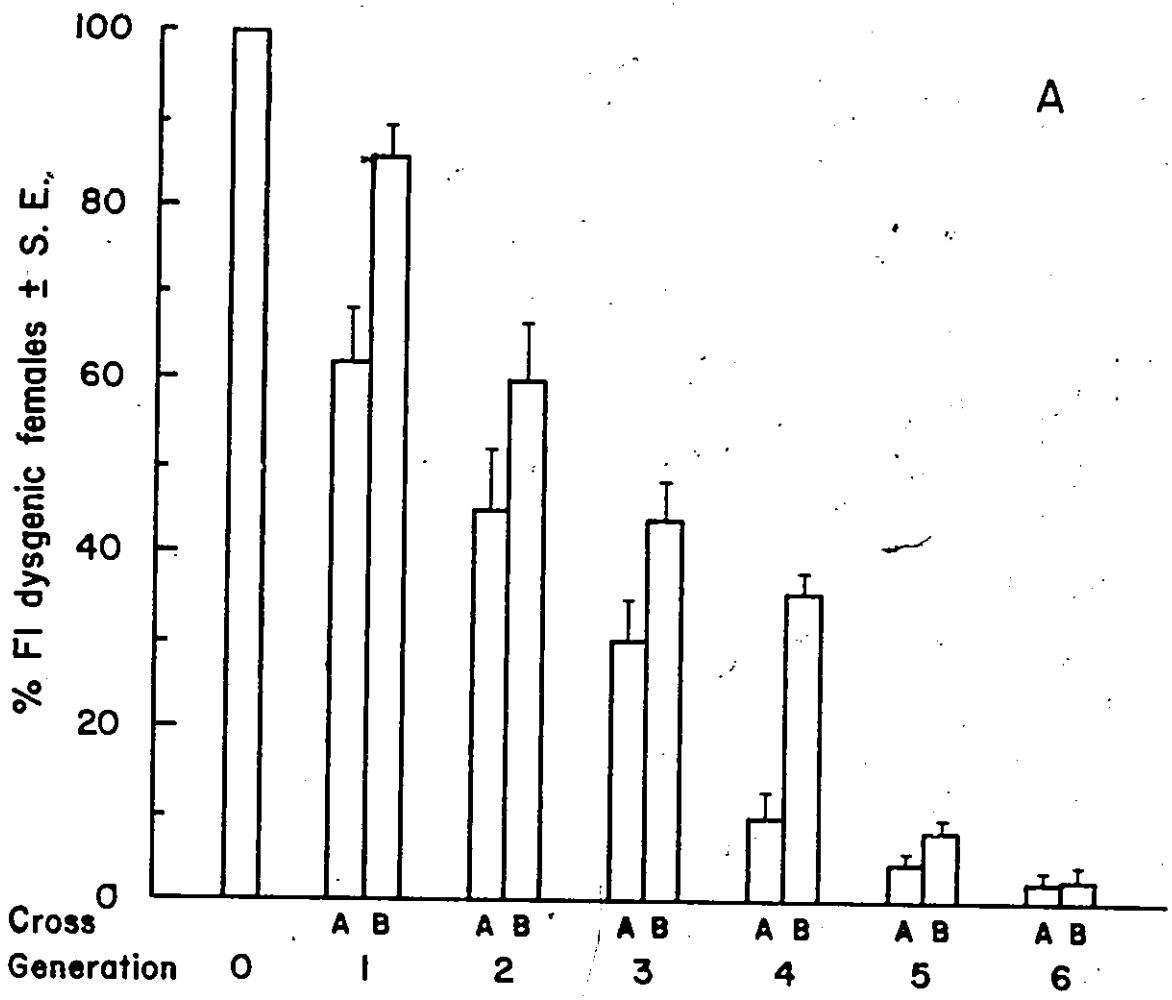
$$\% \text{ P elements} = \frac{\dfrac{\text{HIII densitometer reading}}{\text{XDH densitometer reading}} \text{ (for experimental stock)}}{\dfrac{\text{HIII densitometer reading}}{\text{XDH densitometer reading}} \text{ (for Harwich stock)}} \times 100$$

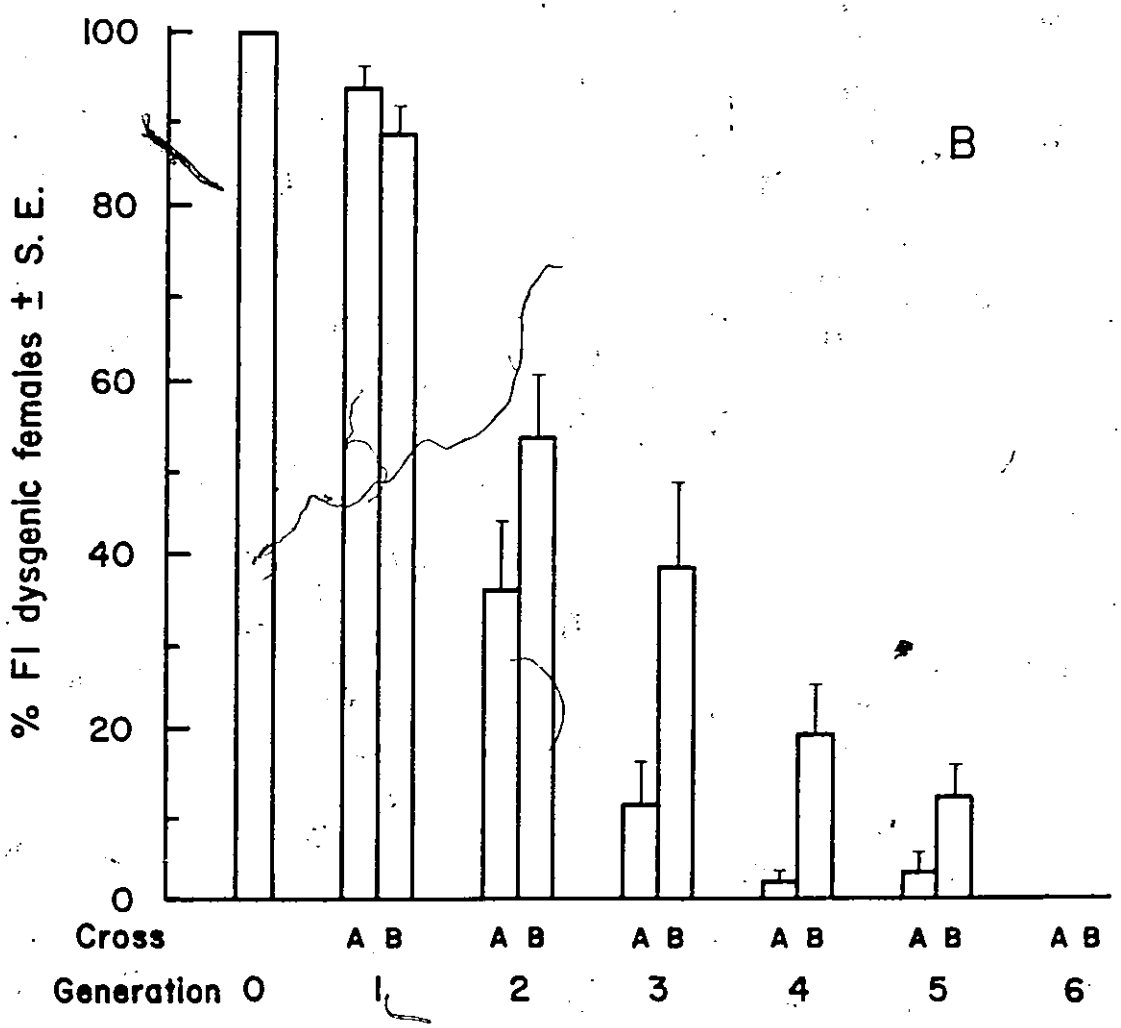
Because DNA samples from the Harwich strain were included on all filters as a standard this method prevents variables such as amount of probe, radioactive intensity of the probe and exposure times from having any effect on the final calculation of the percentage of P elements.

Results

The effect of continual backcrossing to M-type flies on the P element activity of males at 27°C, 24°C and 21°C is shown in Figures 2.3 a,b and c respectively. The generation 0 cross was a Harwich male x Canton-S female control, indicating 100% dysgenesis in this cross. At all three experimental temperatures, the ability of males to induce dysgenesis decreased rapidly, until by generation 6 it was below 5%, which is generally considered background (Engels, 1979). The effect of the three variables, type of backcross, experimental temperature and number of backcross generations were investigated by three way analysis of variance (ANOVA). All main variables and interaction effects were found to be significant at $p \leq 0.01$, with the exception of the type of backcross*temperature effect which was not significant (Table 2.1). Several trends were detected using ANOVA. First, contrary to what was predicted, flies from the control cross had on average a greater dysgenic ability than flies from the dysgenic cross (35.9% versus 22.1%). Second, there was a significant increase in dysgenic potential of flies with increasing temperature (Table 2.2). However, most of the non-error variation (60% of 70.1%) was explainable by the effect of the number of generations of backcrossing, i.e., the most obvious effect was the reduction in P activity with each generation of backcrossing regardless of the type of cross or experimental temperature. Differences between the variables, experimental temperature and backcross generation were analysed

FIGURE 2.3 a,b and c: The effect of repeated backcrossing on P element activity at different temperatures. The experimental temperatures used were (a) 27°C, (b) 24°C and (c) 21°C. Cross A is the dysgenic cross whereas Cross B is the control cross. The standard error calculations are based on the three replicate populations from each experiment


A
100
80
60
40
20
0
% FI dysgenic females ± S. E.
Cross
A B
A B
A B
A B
A B
A B
Generation
0
1
2
3
4
5
6


100
80
60
40
20
0
% F1 dysgenic females ± S.E.
B
Cross
A B
A B
A B
A B
A B
A B
Generation 0
1
2
3
4
5
6

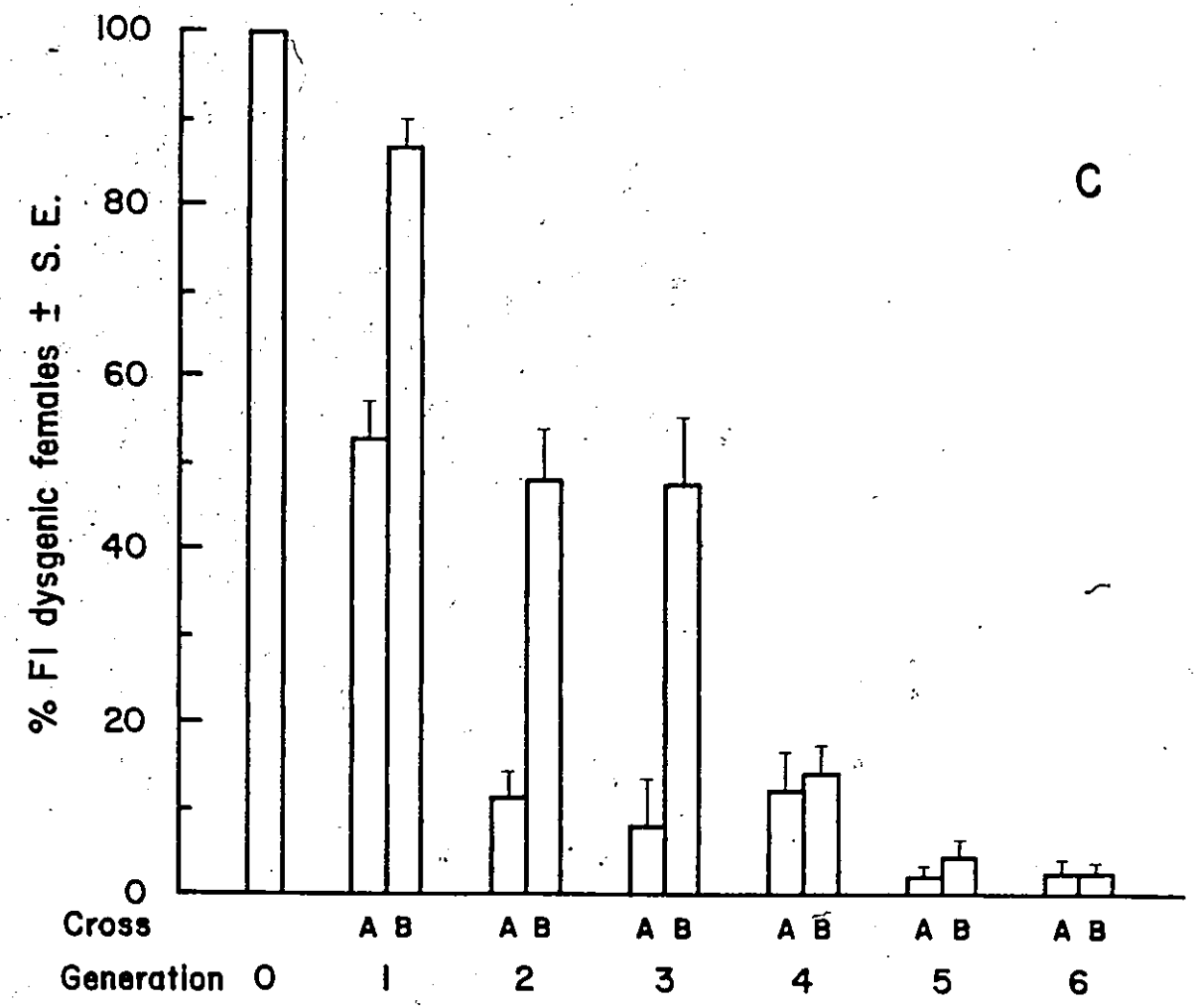

100
80
60
40
20
0
% F1 dysgenic females ± S. E.
C
Cross
A B
A B
A B
A B
A B
A B
Generation 0
1
2
3
4
5
6

TABLE 2.1: Three way analysis of variance of P element activity -- in backcross experiments.

TREATMENT	D.F.	SUM OF SQUARES	F VALUE
Type of Backcross (A)	1	257.6	73.25 ***
Temperature (B)	2	62.6	8.89 ***
Backcross Generation (C)	5	3676.7	209.05 ***
A X B Interaction	2	13.6	1.94 N.S.
B X C Interaction	5	102.3	5.82 ***
A X C Interaction	10	145.3	4.13 ***
A X B X C Interaction	10	102.5	2.91 **
Error	504	1772.8	

*** $p \leq 0.001$ ** $p \leq 0.01$

D.F. = degrees of freedom

using a Duncan's multiple range test as illustrated in Table 2.2. The P element activity at 27°C and 24°C did not differ significantly, however both had significantly higher P element activity than at 21°C. Generations 1 through 5 had P element activities that decreased significantly each generation, however there was no significant difference between generations 5 and 6 in P element activity (Table 2.2).

In order to determine whether the decrease in gonadal dysgenesis in each generation was correltaed with a decrease in the number of P elements, DNA from backcross generations 3 and 6 was probed with both an XDH (Xanthine dehydrogenase) and a P element probe, and the percentage of P elements in each generation calculated in comparison with the control strain (Harwich). Figure 2.4 illustrates a dot blot with Harwich and generations 3 and 6 probed with the XDH and HIII probe. We can see that the amount of DNA on the filter, (as determined by the XDH probe), is greater for the F6 than the F3 generation samples; however the P element (HIII) signal for the F6 generations is much lower. The Harwich dilution series show a decrease in signal with decreasing concentration for both probes, but the HIII probe produces a much stronger signal. Table 2.3 summarizes the data from the experimental crosses performed at 27°C, 24°C and 21°C. A more detailed account of this data is provide in Appendix 2.1. By the F3 generation there was a substantial decrease in the number of P elements. In the F6 generation there was a further reduction in P element copy number, but the

FIGURE 2.4: The measurement of P element copy number using DNA hybridization. F3 and F6 refer to DNA samples from backcross generations 3 and 6. A1 and A2 are replicates from the dysgenic cross (A), B1 and B2 replicates from the control cross (B). XDH is a single copy gene probe whereas HIII is a probe specific to the P element. As a control, serial dilutions of DNA from the Harwich P strain are included. See text for details.

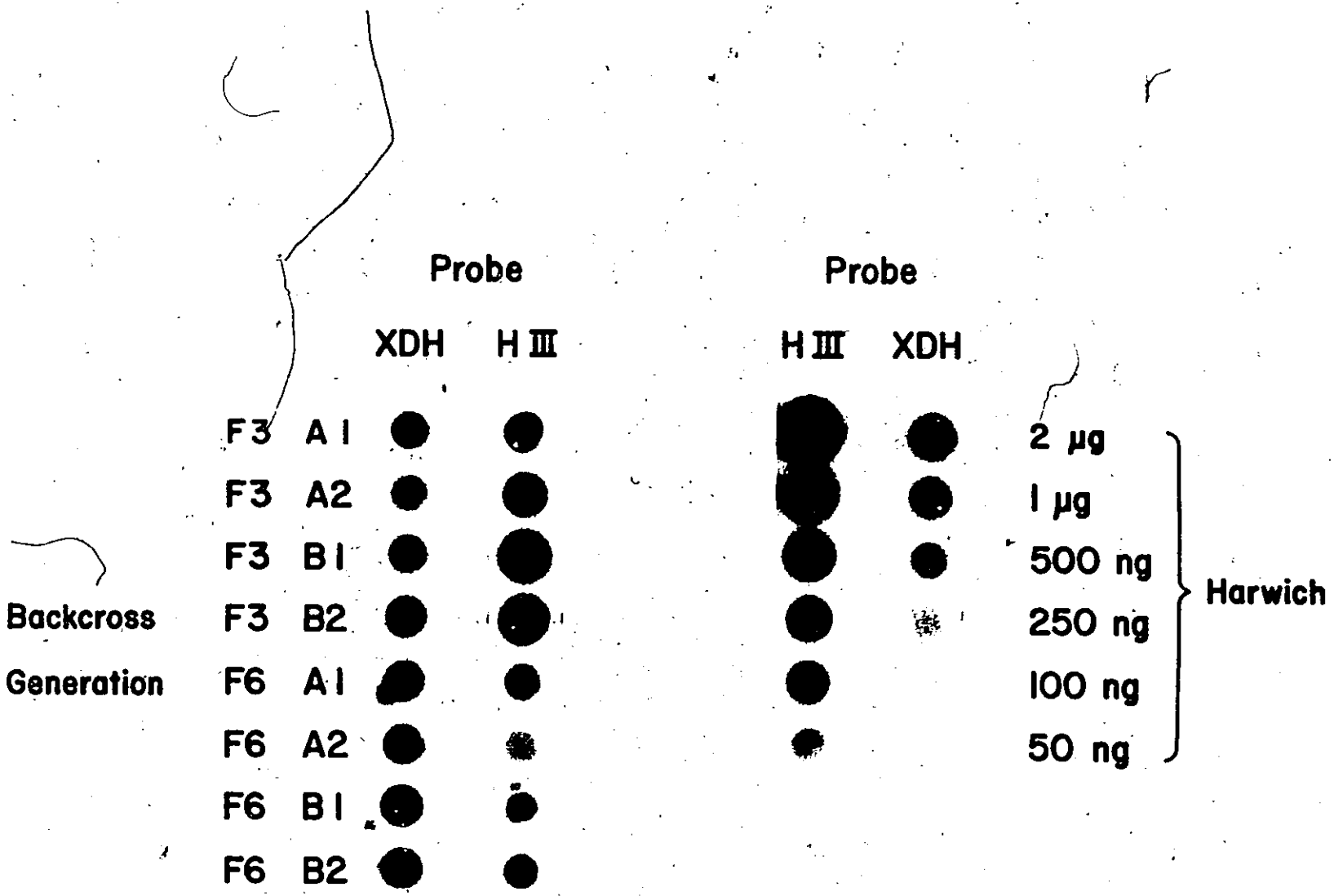

Probe
XDH
H III
F3 A1
F3 A2
F3 B1
F3 B2
F6 A1
F6 A2
F6 B1
F6 B2
Backcross Generation
Probe
H III
XDH
2 µg
1 µg
500 ng
250 ng
100 ng
50 ng
Harwich

TABLE 2.2: Duncan's multiple range test of P element activity for the variables (a) experimental temperature and (b) backcross generation.

(a) Temperature

Temperature	Mean	Grouping*
27oC	32.83%	A
24oC	29.56%	A
21oC	24.56%	B

(b) Generation

Generation	Mean	Grouping*
1	78.9%	A
2	42.0%	B
3	28.9%	C
4	16.2%	D
5	6.0%	E
6	1.9%	E

* Means with the same letter are not significantly different.

TABLE 2.3: Percentage P element sequences compared to the Harwich stock using DNA hybridization, for experimental crosses at 27oC, 24oC and 21oC. (For calculation method, see Materials and Methods).

	Temperature		
Generation	27oC	24oC	21oC
F3 A1	13.4%	13.0%	12.2%
F3 A2	15.3%	14.3%	12.7%
F3 B1	24.7%	15.1%	13.5%
F3 B2	16.6%	15.4%	13.5%
F6 A1	12.9%	8.4%	2.0%
F6 A2	10.1%	13.8%	1.7%
F6 B1	11.8%	13.2%	4.5%
F6 B2		10.6%	2.4%

Note: A1 and A2 are replicate populations from the dysgenic cross A. B1 and B2 are replicate populations from the control cross B.

decrease at 27°C and 24°C was less than expected based on the decrease in gonadal dysgenesis measurements. This may indicate that there was some transposition occurring at 27°C and 24°C (as indicated in Table 2.4) maintaining the P element copy number, or may simply be due to experimental error. The relationship between P element activity and P element copy number as measured by DNA hybridization is shown in Figure 2.5. While there is an increase in P element activity with increasing P element copy number, this relationship is quite weak.

Two types of selection might be acting on P elements or P element-carrying chromosomes. First, there may be negative selection against the F1 offspring of a cross if that cross is dysgenic. In order to monitor the negative selection resulting from dysgenic offspring, in each generation experimental females were screened for evidence of gonadal dysgenesis. The results of these tests are shown in Table 2.4. As expected, the F1 offspring of the Harwich male X Canton-S female showed high levels of sterility at 27°C but this level of sterility decreased with each subsequent generation of backcrossing. Lower levels of sterility were detected at 24°C whereas at 21°C there was no detectable gonadal dysgenesis in any of the generations. In contrast, in the Control cross (Harwich females X Canton-S males) there was less that 5% gondal dysgenesis in any of the backcross generations at either temperature, except generation 5 and 6 where some dysgenesis seemed to occur at 27°C and 24°C. Second, there could be positive or negative selection for P

FIGURE 2.5: The relationship between P element activity and P element copy number as measured by DNA hybridization. The equation of the line of best fit is;

$Y = 8.99 + 0.174X$

$r = 0.646$

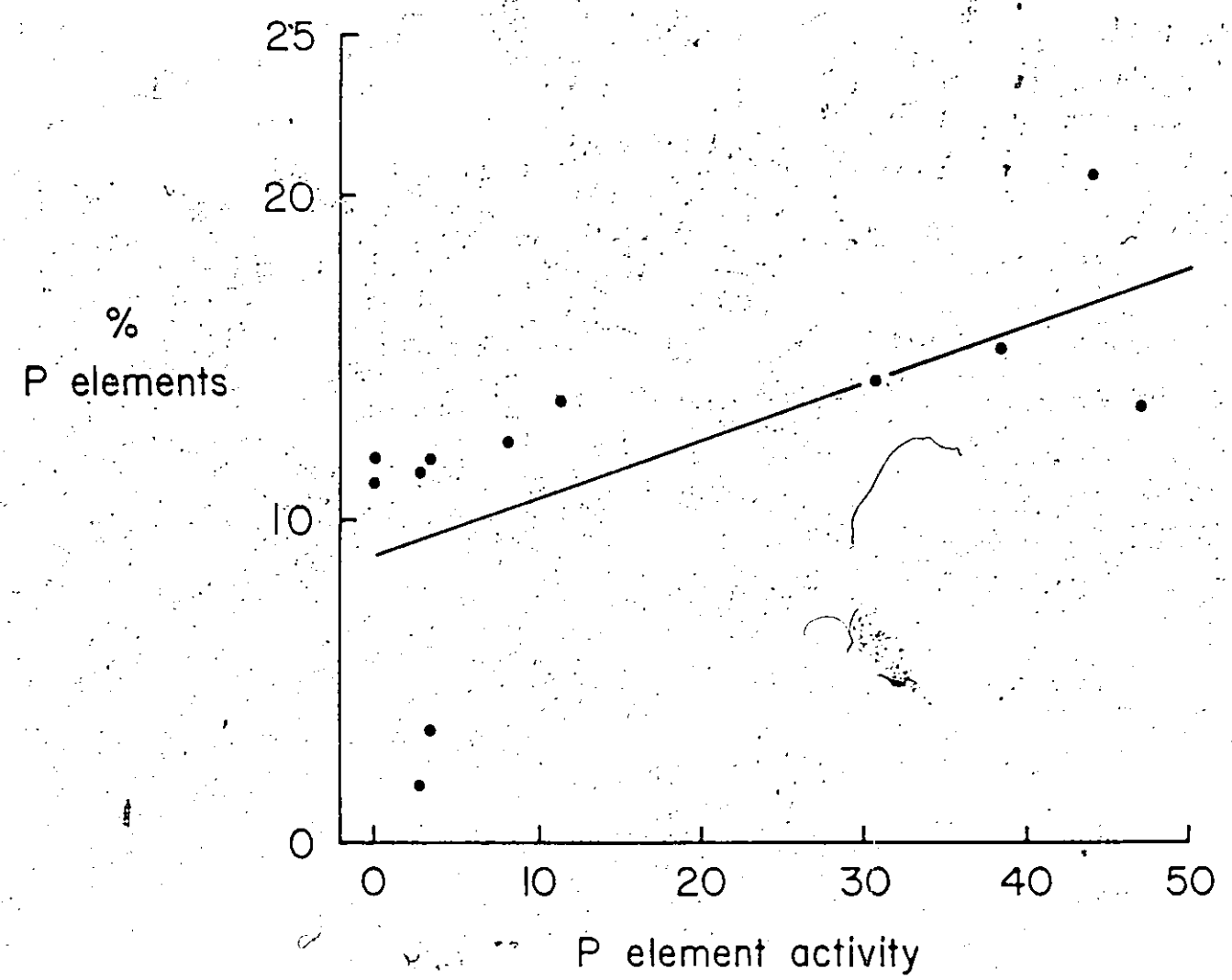

25
20
10
0
%
P elements
0
10
20
30
40
50
P element activity

TABLE 2.4: Frequency of dysgenic females in each backcross generation in Experiments A and B (n=60).

Generation	Exp. A Temperature			Exp. B Temperature		
	27oC	24oC	21oC	27oC	24oC	21oC
1	90%	48%	2%	3%	2%	0%
2	53%	7%	0%	2%	0%	0%
3	15%	0%	2%	5%	0%	2%
4	8%	0%	4%	3%	2%	3%
5	7%	5%	0%	17%	8%	0%
6	3%	0%	0%	8%	0%	0%

elements because of selection for P element carrying chromosomes through a hitch-hiking effect. This was monitored by taking advantage of the fact that the Harwich strain used had a white eye colour. In the control cross (Harwich male X Canton-S female) the F1 male offspring should have white eyes, and the frequency of white eyed F1 males will reflect the frequency of Harwich X chromosomes in the previous generation. Table 2.5 illustrates the effect of continual backcrosses on the frequency of white-eyed males in each generation. As one would predict based on there being no strong positive or negative selection against the Harwich X chromosome, the frequency of white eyed males decreased by approximately 50% each generation. The only deviations were in generations 5 and 6 where sampling error, resulting from the small population size, probably influenced the results.

TABLE 2.5: Percentage of white eyed males in each generation during backcross experiment A (n=100).

Generation	Temperature 24oC	Temperature 21oC	Theoretical % No selection
1	100%	100%	100%
2	50.3%	48.8%	50%
3	24.4%	23.7%	25%
4	13.4%	13.8%	12.5%
5	3.2%	2.0%	6.2%
6	1.4%	1.3%	3.1%

Discussion

It has been shown through these experiments that the transposition rate of P elements is not sufficient to overcome the 50% selective pressure that results from continuous backcrossing, and these elements are therefore not capable of maintaining their normal level of 30-50 copies per genome. These results are contrary to what has been found in population cage studies, where even with the selective pressure induced by high temperature sterility, most populations evolved to the P-type (Kidwell et al., 1981; Kiyusa & Kidwell, 1984; Chapter 3).

This reduction in P elements was demonstrated both as a reduction in P element activity and a reduction in P element copy number. While there was a correlation between P element activity and P element copy number, this correlation was rather weak. This weak correlation primarily occurs when there is less than 12% of wild-type (Harwich) P element copies (4 to 6 copies). It may well be that the correlation between dysgenic potential and copy number is much stronger if there are higher levels of P elements. Bingham et al. (1982) found very similar results. Below four P element copies there was very little correlation between copy number and percent sterility (P element activity). However there was a much stronger correlation once there were more than 4 copies contributing to the dysgenic potential. Once there are reduced levels of P elements, presumably some flies have several active P factors whereas other flies have deleted P elements which cannot induce dysgenesis. Thus it is not

suprising that there is a weak correlation at low P element copy numbers.

As mentioned breifly in the Materials and Methods section, the use of the HIII P element fragment allows one to measure the change in P element copy number, but does not provide any information on whether there has been a change in the relative proportion of P factors to P elements. It is possible that with subsequent generations, this ratio would change, and this could have important implications on the rate of spread of these elements. While recognizing that this is an important issue, it was felt that this represents a further question, one that follows the question of the spread or loss of P elements.

Contrary to what was predicted , the dysgenic cross had, on average, lower P element activity than the control cross. This could occur for two reasons. First, selection against dysgenic hybrids might have resulted in lower rates of P element spread in this cross. However, if this were the case, one would also expect the higher temperature experiments to have lower rates of P element activity, the reverse of what was found. Second, the greater P element activity in the control crosses could result from the experimental design. Since the dysgenic cross (P males X M females) produces males with an M-type X chromosome, they would be expected to have lower P element activity than the control cross (P females X M males) males which have a P-type X chromosome.

Kidwell et al. (1981) and Kiyusa and Kidwell (1984)

concluded that the increase in P flies in a population is unlikely to result from positive selection for P-carrying chromosomes. Similarly there was no evidence of positive or negative selection for P chromosomes as the frequency of white eyed males was identical to what would be predicted based on no selection. The only case where this was not true was in generations 5 and 6 , but here low frequencies of the white eyed marker resulting from sampling error would explain the results.

In another eukaryotic system, Alleman and Freeling (1986) studied plants of a strain containing 10-15 copies of Mu, a transposon of maize. They found that when Mutator plants (containing the transposon) were crossed to plants lacking Mu, the progeny plants had approximately the same number of Mu sequences as did their Mutator parents. Approximately one half of these copies had segregated from their parent and one-half had arisen by transposition and integration into new positions in the genome. This maintenance of Mu element copy can be accounted for by a transposition rate that can double the number of Mu copies each generation. Thus Mu differs from the P element in being able to maintain its copy number through single backcrosses, whereas there are clearly some conditions under which P elements will not be able to maintain its copy number in a populations.

The experiments outlined in this chapter were designed to model the introduction of a small number of P-type flies into a large M population. However, since male or female from each generation are continuously backcrossed to M-type flies, this in

effect models the introduction of P-type flies into an infinitely large M population. Thus the rapid loss of P elements suggests that the rate of spread of P elements may be determined in part by migration rates and population sizes of Drosophila populations in nature.

CHAPTER 3

THE SPREAD OF P ELEMENTS IN MIXED P-M POPULATIONS

Introduction:

In the experiments described in Chapter 2, repeated backcrossing of a P-type strain to an M-type strain resulted in a reduction in the number of P elements in each subsequent generation. This reduction in the number of P elements could be seen both in terms of P element copy number, as measured by DNA hybridization and by the commonly used test of gonadal dysgenesis. However, these experiments may not accurately model what is happening in nature, since the breeding scheme is specified, whereas in nature flies will breed randomly with respect to P and M type (Kidwell and Novy, 1979).

The rationale for the experiments detailed in this Chapter was to model the introduction of a small number of P flies into a large M-type population, and to see if under conditions of random breeding these elements can increase in frequency as a result of replicative transposition. These experiments are similar to those of Kidwell et al. (1981) and Kiyasu and Kidwell (1984) who studied the ability of P elements to survive and spread in mixed P-M populations. Kidwell et al. (1981) found that at 20°C, a condition where there was no negative selection against P elements, mixed P-M populations change unidirectionally towards the P-type fairly rapidly. Under conditions of strong negative sterility selection, Kiyasu and Kidwell (1984) found that most mixed populations evolved to the P-type, however the equilibrium

level of P element activity was lower than in the earlier experiments at 20°C (Kidwell et al. 1981). Kidwell (1986) introduced P elements into M strain genomes and then studied their evolution in population cages. She found that a number of the lines rapidly increased their P element activity to close to 100% maximum within 10 generations. The cytotype of these lines remained M-type until the time when maximum P element activity was reached, after which a rapid switch to P cytotype was observed. Kidwell (1986) used restriction enzyme analysis to confirm the presence of multiple copies of new P elements.

The purpose of these experiments was to determine if P elements could spread in an M-type population, when starting from very low initial frequencies (1% and 10%). Second, by using the technique of DNA hybridization to measure P element copy number (Chapter 2), can one distinquish between an increase in P-type flies in a population as a result of chromosomal segregation versus an actual increase in P element copies?

Materials and Methods

Stocks:

(1) Harwich, (2) Canton-S. See Chapter 2 for detailed descriptions of these stocks.

(3) π_1 : An inbred wild-type P strain originally collected from Madison, Wisconsin by W.R. Engels (Engels, 1979).

Population Cages: All population cage experiments were carried out at 21°C and raised on Yeast Agar media (900 mls H_2O, 100 gms sugar, 50 gms dried yeast, 15 gms agar). Each population was maintained in 2, 250ml glass jars, the flies from both jars were mixed each generation and approximately 400 adult flies were transferred to jars containing fresh food. The change in food media from Carolina Instant Drosophila media was necessary since the instant media would not remain in the jars during the transfer of flies. Four different populations were initiated with varying proportions of P and M strains, with four replicates of each population. Table 3.1 outlines the starting frequencies for each population. For populations A and B, Canton-S females were mated to P males, and these females were used to start the population cage. This approach was chosen to reduce the chance that random genetic drift might reduce or eliminate the number of P-type flies in early generations. Therefore in the initial generation, there were actually 2% Canton-S females which had been mated to Harwich males. In all populations, flies were transferred as discrete generations to fresh population cages.

In generations 5, 10 and 20 each population was tested for P

TABLE 3.1: Starting frequencies for population cage experiments.

Population	Type of Experiment	Starting Numbers			
		Harwich ♀'s (P)	π_2 ♀'s (P)	CS/P* ♀'s	Canton-S ♀'s (M)
A	1%			8	392
B	1%			8	392
C	10%	20			180
D	10%		20		180

* The CS/P females were from either Harwich ♂'s X Canton S ♀'s (Population A) or π_2 ♂'s X Canton S ♀'s (Population B).

element activity and P cytotype. In generations 2, 5, 10 and 20, flies were frozen and their DNA extracted to measure P element copy number.

Method of P element activity determination: Individual males were mated with 3-4 Canton-S females, the F1 hybrid females were aged 2-4 days and their ovaries dissected according to the methods of Schaefer et al. (1979). Ten females from each individual mating were scored for sterility and the percentage dysgenesis was calculated as the mean of 5 individual matings.

Method of Cytotype determination: Individual females were mated with 2-3 Harwich males, the F1 hybrid females were aged 2-4 days and their ovaries dissected (Schaefer et al. 1979). Ten females from each individual mating were scored for sterility and the percentage dysgenesis calculated as the mean of 5 individual matings.

Statistical Analysis: Analysis of variance and Duncan's multiple range tests were performed as outlined in Chaptgr 2. The analysis of variance of cytotype was done slightly differently than for P element activity. Analysis of variance makes several assumptions; that samples are randomly chosen with a normal distribution and equal variances (Sokal and Rohlf, 1969). Since several of the replicate populations had 100% M cytotype in generation 5, all the replicates were grouped together in a two-way ANOVA to ensure that each cell had roughly equal variances.

Detection of P elements in crosses by hybridization: The presence of P element sequences in a particular generation was investigated by DNA hybridization. DNA was extracted from 100-500 flies and dot blots were performed as outlined in Chapter 2. However, in these experiments replicate blots were probed with XDH and HIII rather than reprobing a washed blot, since using replicate blots proved to be much quicker and easier to do. Preliminary experiments had demonstrated that both techniques yeilded very similar results. The percentage of P elements in a particular generation was measured as the ratio of the signal from a single copy gene probe (XDH) to that from a P element probe (HIII) as outlined in Chapter 2.

Results

The evolution of P element activity in the population cages is shown in Figure 3.1. The population cages started with 1% and 10% P flies rapidly evolved to P-type populations in all replicates. A more complete account of the P element activity and cytotype data is presented in Appendix 3.1. It is interesting to note that the increase in P element activity occurred more slowly in the 10% population started with than in either of the other three populations. Changes in P element activity were also analysed using analysis of variance (ANOVA). As illustrated in Table 3.2 the main effects, generation and experiment were highly significant, while the generation X population effect was slightly significant. All other effects were not significant (Table 3.2). Differences between generations and experiments were analysed using a Duncan's multiple range test (Table 3.3). All three generations were found to differ significantly from each other with generation 5 having the lowest P element activity followed by generation 10 and generation 20 having the highest activity. Experiments C and B were found to have the highest activity while experiments D and A were significantly lower in overall activity than C or B.

Figure 3.2 demonstrates the change in cytotype in the population cages at generations 5, 10 and 20. In all four experiments the change from M to P cytotype occurred much later than did the P element activity, however once the shift in cytotype began, it occurred more rapidly than the change in P

FIGURE 3.1: Changes in P element activity at Generations 5, 10 and 20 for the 4 population cage experiments.

The experimental starting frequencies were,

A = 1% Harwich X 99% Canton-S

B = 1% $_2$ X 99% Canton-S

C = 10% Harwich X 90% Canton-S

D = 10% $_2$ X 90% Canton-S

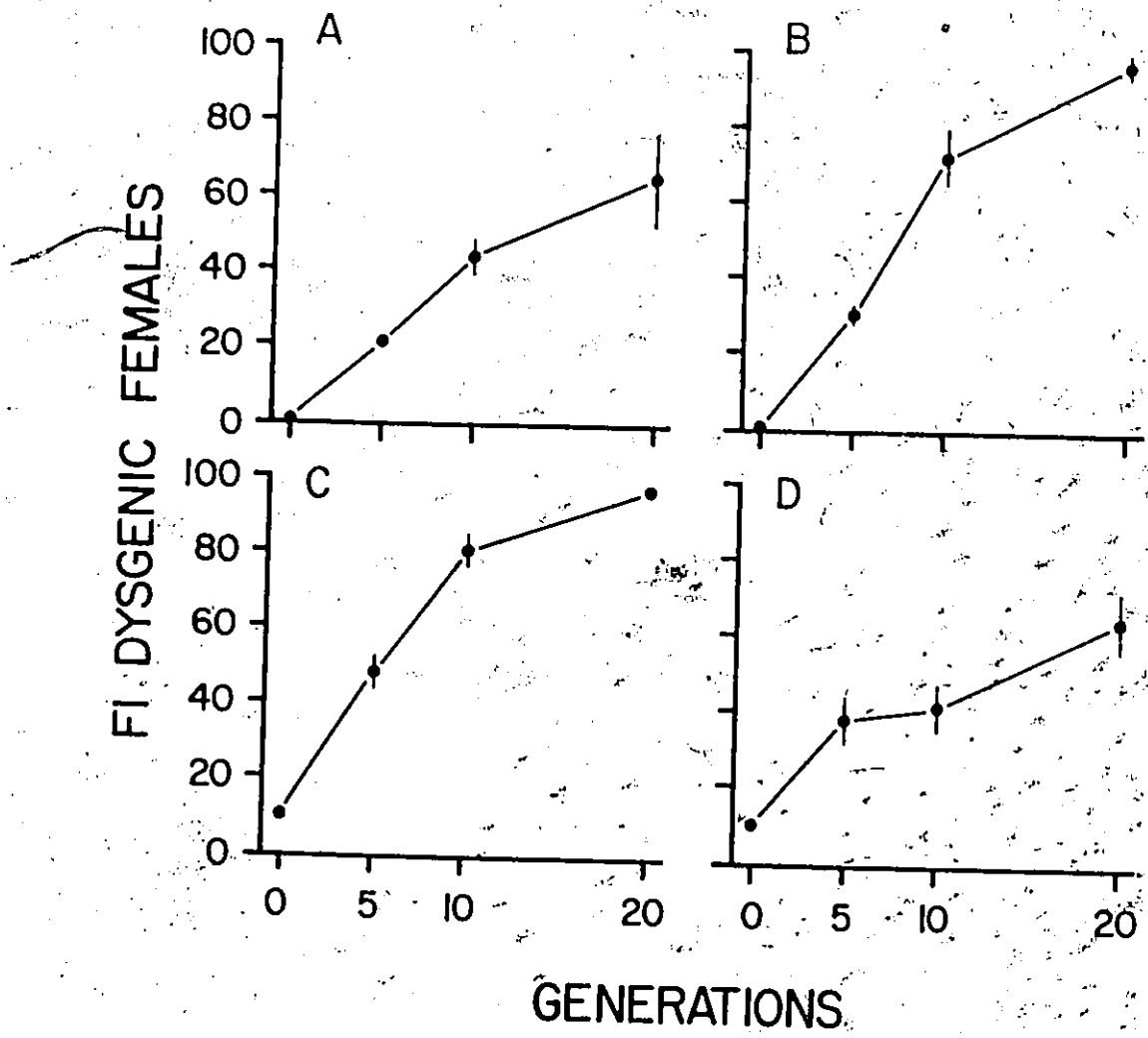

A
B
C
D
100
80
60
40
20
0
0
5
10
20
F1 DYSGENIC FEMALES
GENERATIONS

TABLE 3.2: Three way analysis of variance of P element activity in the population cage experiments.

TREATMENT	D.F.	SUM OF SQUARES	F VALUE
Generation (A)	2	654.7	31.6 ***
Experiment (B)	3	356.8	11.5 ***
Population (C)	3	23.3	0.7 N.S.
A X B Interaction	6	105.2	1.7 N.S.
B X C Interaction	6	112.4	1.8 *
A X C Interaction	9	126.3	1.4 N.S.
A X B X C Interaction	18	202.6	1.1 N.S.
Error	192	1985.2	

*** $p \leq 0.001$ * $0.1 \leq p \leq 0.05$

D.F. = degrees of freedom

N.S. = Not significant

TABLE 3.3 Duncan's multiple range test of P element activity for generation and experimental effects.

Generation

Generation	Mean	Grouping*
20	6.69	A
10	5.21	B
5	2.69	C

Population

Experiment	Mean	Grouping*
C	6.50	A
B	5.53	A
D	3.98	B
A	3.43	B

* Means with the same letter are not significantly different

element activity. By the fifth generation there had been very little change in cytotype, however by generation 10 three of the four experiments had begun to switch cytotype. By generation 20 most of the population cages had below 20% M cytotype. Changes in cytotype were also analysed using analysis of variance. Only the effect of generation was found to be significant (Table 3.4). Differences between generations were analysed using a Duncan's multiple range test. Generations 5 and 10 did not differ significantly in their cytotype, however the flies from generation 20 had on average a lower M cytotype (Table 3.5).

In order to determine whether the change in P element activity and cytotype over time was correlated with an increase in the number of P elements, DNA from backcross generations 2, 5, 10 and 20 was probed with both an single copy (XDH) and a P element (HIII) probe and the percentage of P elements in each generation calculated in comparison with the control strain (Harwich). Figure 3.3 illustrates a dot blot from Population A (1% Harwich X 99% Canton-S) showing Harwich and generations 2, 5, 10 and 20 probed with the XDH and HIII probe. It can be seen that the amount of signal from the P element probe increased from generation 2 through to generation 20 demonstrating an increase in P element copy number. Table 3.7 summarizes the P element copy number data from the four population cage experiments. In all population cages there was a rapid increase in P element copy number with increasing generations. Appendix 3.2 illustrates several examples of dot blots used in calculating P element copy

FIGURE 3.2: Changes in M cytotype at Generations 5, 10 and 20 for the population cage experiments.

The experimental starting freqencies were,

A = 1% Harwich X 99% Canton-S

B = 1% $_2$ X 99% Canton-S

C = 10% Harwich X 90% Canton-S

D = 10% $_2$ X 90% Canton-S

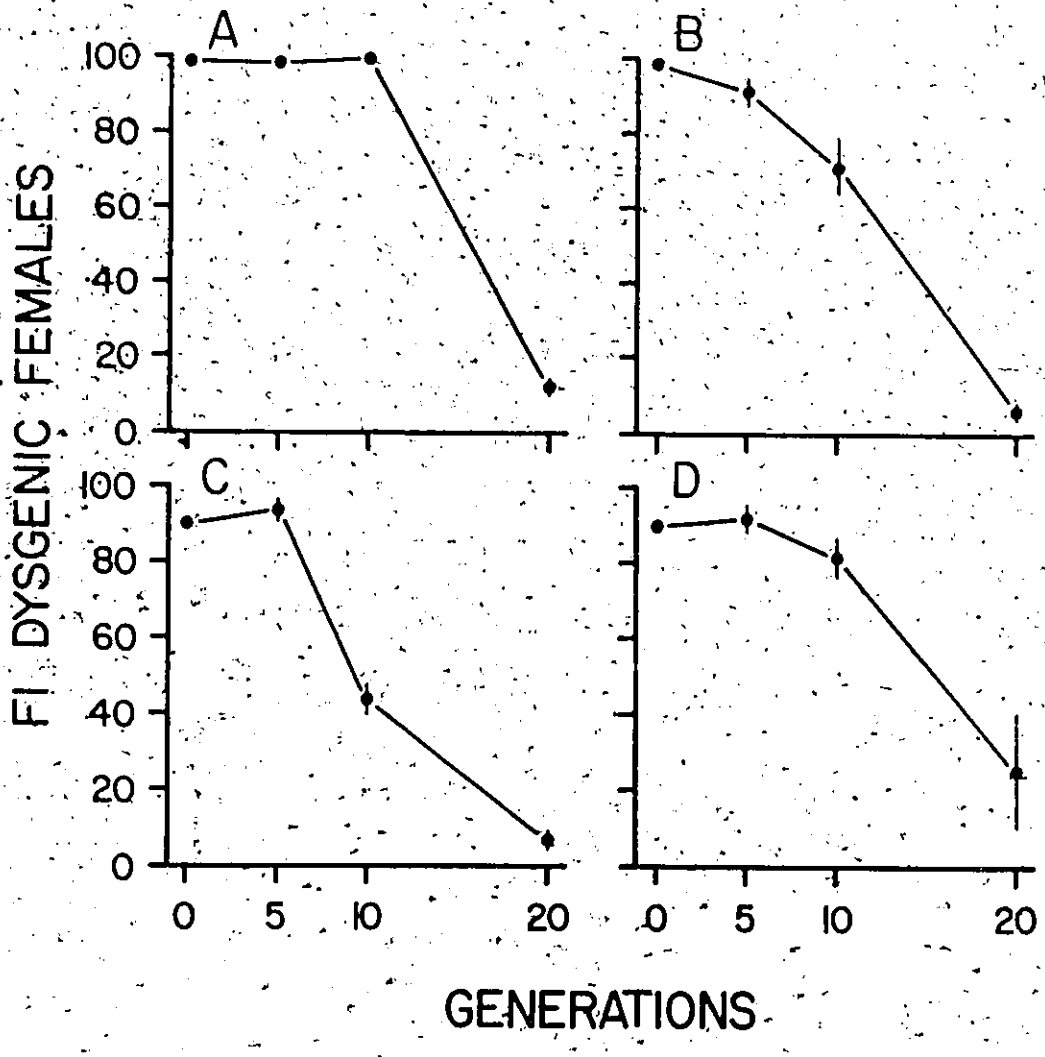

A
B
C
D
100
80
60
40
20
0
0
5
10
20
F1 DYSGENIC FEMALES
GENERATIONS

TABLE 3.4: Two way analysis of variance of cytotype for the population cage experiments.

TREATMENT	D.F.	SUM OF SQUARES	F VALUE
Experiment (A)	3	16.7	0.45 N.S.
Generation (B)	2	158.0	6.31 **
A X B Interaction	6	12.1	0.16 N.S.
Error	228	2817.1	

** $p < 0.01$

D.F. = degrees of freedom

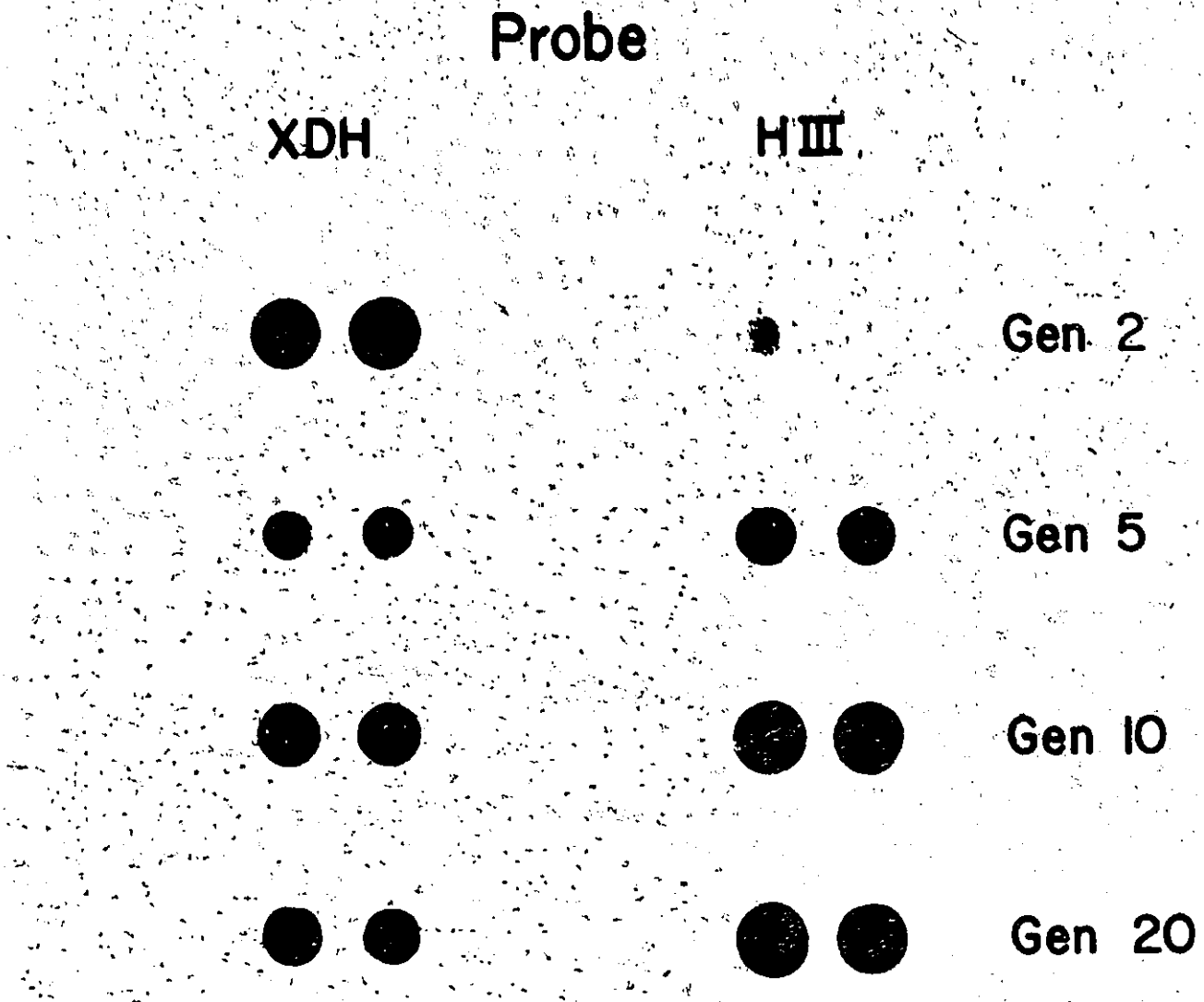
N.S. = Not significant

TABLE 3.5 Duncan's multiple range test of cytotype for the different generations.

Generation	Mean	Grouping*
5	2.91	A
10	2.71	A
20	1.10	B

* Means with the same letter are not significantly different.

FIGURE 3.3: The measurement of P element copy number using DNA hybridization. The DNA is from Population A (Harwich X Canton-S; 1%) replicate cages 1 and 2 and has been probed with a single copy gene probe (XDH) and a P element probe (HIII). For the details of copy number calculations see Materials and Methods.


Probe
XDH
HIII
Gen 2
Gen 5
Gen 10
Gen 20

number, whereas a more detailed account of the data presented in Table 3.6 is presented in Appendix 3.3. The relationship between the increase in P element copy number and P element activity is shown in Figure 3.4. The slope of the line of best fit is 0.816, suggesting that there is nearly a 1:1 relationship between P element activity and P element copy number. Moreover, the correlation coefficient ($r = 0.851$) indicates that most ($r^2 = 0.724$) of the change in P element activity can be explained by an increase in P element copy number in the population.

Two types of selection might be acting on P elements or P element-carrying chromosomes. First, there may be negative selection against the F1 offspring of a cross if that cross is dysgenic. However, in the P-M system there is no detectable gonadal dysgenesis at 21oC (Kidwell, 1979; Chapter 2). Second, there could be positive or negative selection for P elements because of selection for P element-carrying chromosomes through a hitch-hiking effect. This was monitored by taking advantage of the fact the Harwich strain used had a white eye colour. Therefore, the frequency of white eyed males in any generation will reflect the frequency of Harwich X chromosomes in the previous generation. Table 3.7 illustrates the white-eyed males in each generation from 1 to 10. In populations A and C there was an increase in the percentage of white eyed males, up to generation 4, but their frequency then began to drop. These results suggest that although there may have initially been some selection for P chromosomes in the first four generations, after

TABLE 3.6: Percentage P element sequences compared to Harwich using DNA hybridization, for population cage experiments. Samples 1 and 2 from each population are replicate population cages from within one experiment.

Generation	Population		Starting Frequency 1%	Starting Frequency 10%
2	Harwich	1	3.5%	9.8%
		2	2.5%	3.1%
	π_1	1	9.2%	8.9%
		2	7.1%	7.7%
5	Harwich	1	27.9%	18.1%
		2	30.1%	12.4%
	π_1	1	20.3%	24.2%
		2	29.2%	13.4%
10	Harwich	1	25.1%	71.5%
		2	31.1%	68.9%
	π_2	1	53.4%	42.0%
		2	59.1%	41.0%
20	Harwich	1	59.4%	73.1%
		2	60.7%	73.1%
	π_2	1	89.8%	84.4%
		2	86.0%	71.0%

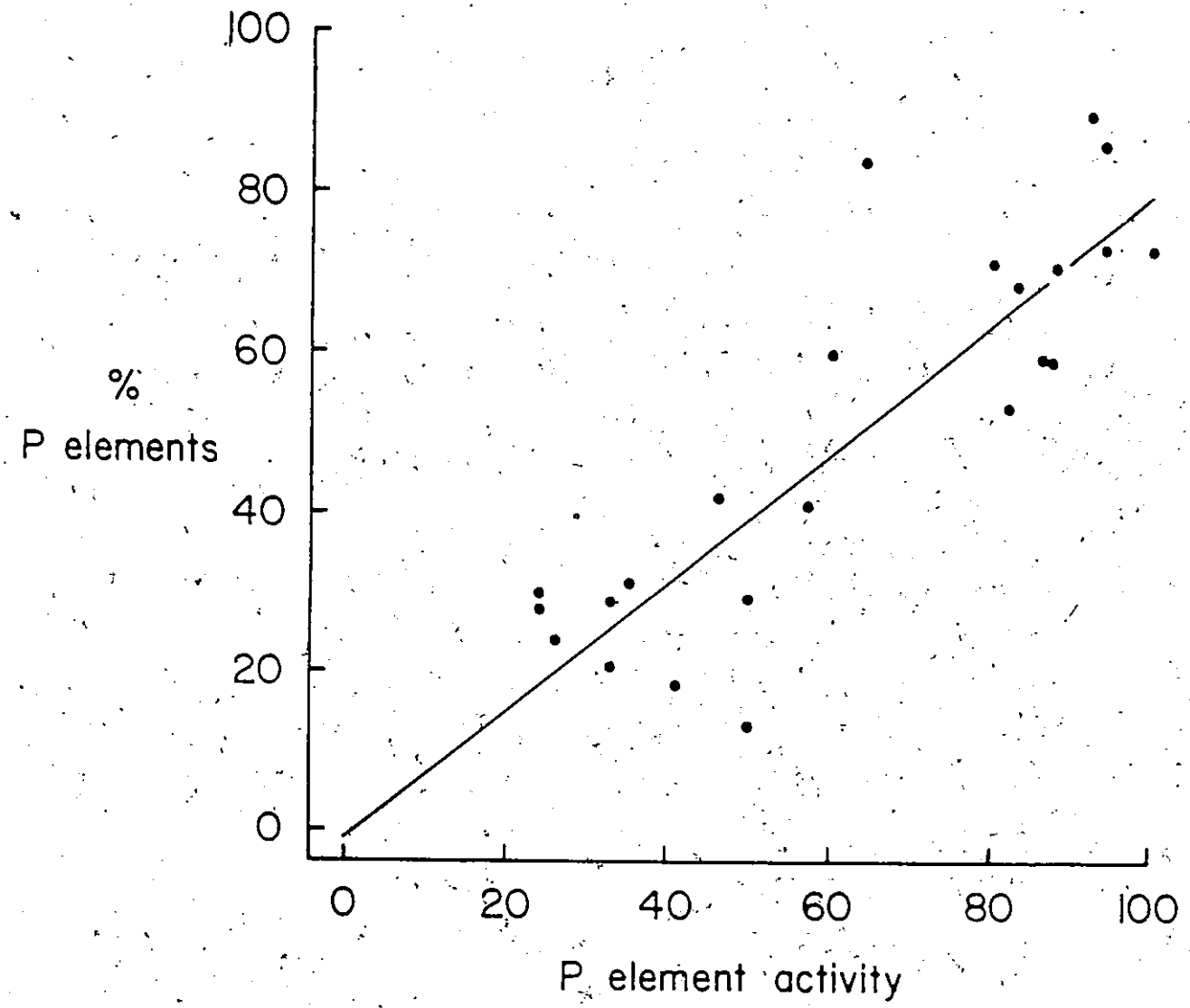
Note: Harwich 1% is the same as Population A (Figure 3.2, 3.3)
1% is the same as Population B (Figure 3.2, 3.3)
Harwich 10% is the same as Population C (Figure 3.2, 3.3)
10% is the same as Population D (Figure 3.2, 3.3)

FIGURE 3.4: The relationship between P element activity and P element copy number in the population cage experiments.

The equation of the line of best fit is:

$Y = -1.695 + 0.816X$

$r = 0.851$


100
80
60
40
20
0
%
P elements
0
20
40
60
80
100
P element activity

that there did not appear to be any selection acting on the P element carrying chromosomes. This does not completely eliminate the possibility of selection for P element-carrying chromosomes since the white eye marker is at the tip of the X chromosome. Therefore recombination could result in selection for P element carrying-chromosomes not being matched by an increase in the white eye marker.

TABLE 3.7: Percentage of white eyed males in each generation during population cage experiments (X + S.E., n =4).

Generation	Population A	Population C
Initial %	1%	10%
1	1.7% + 0.1	7.1% + 0.8
2	2.5% + 0.6	12.4% + 1.1
3	2.9% + 1.0	9.7% + 1.2
4	3.0% + 1.0	15.1% + 2.8
5	1.6% + 0.3	11.1% + 2.0
6	1.7% + 0.9	5.7% + 0.7
7	1.8% + 0.4	11.0% + 1.5
8	1.4% + 0.7	4.4% + 0.8
9	1.4% + 0.3	5.0% + 0.9
10	1.2% + 0.3	2.1% + 0.1

Discussion

These experiments provide strong evidence that even with very low starting frequencies P elements can spread rapidly through mixed P-M populations. Second, these experiments clearly demonstrate that the increase in P-type flies seen in mixed P-M populations (Kidwell et al., 1981; Kiyasu and Kidwell, 1984; Kidwell, 1986) is closely matched by an increase in P element copy number and is not merely a result of chromosomal contamination.

During the first 5 generations, the increase in P element activity in the 1% populations approached almost a 100% increase each generation. This may in part due to selection for Harwich chromosomes in the first few generations (Table 3.7), however, after generation 4 there was no evidence that this sort of selection was causing an increase in P-type chromsomes. The P element activity of the 10% population cages did not increase as rapidly as in the 1% cages. This was particularly noticeable in the 2 population cage which increased from 10% to 38% by generation 5 but had increased only to 42% P element activity by generation 10. This could be most easily explained by these cages rapidly developing a P cytotype, thereby preventing spread of the P element. However, the cytotype measurements did not indicate that this kind of a change was occurring.

Although by generation 20, populations A through D had all developed a P cytotype, in comparison with the P element activity, the change in cytotype occurred much later, usually

between generations 10 and 20. The cytotype tended to remained M-type until high levels of P element activity had been reached. However when cytoytpe did change, this change occurred very rapidly. Kidwell and Novy (1979), Kidwell (1986) and Engels (1981) found that M cytotype will often be retained for some time in the presence of P elements, but eventually this activity will change. From an evolutionary viewpoint, this system makes sense. P elements cannot spread in a population with a P cytotype, therefore it is in the best interest of the element to first spread by transposition and then produce a P cytotype to prevent over-replication.

Kidwell et. al. (1981) and Kiyasu and Kidwell (1984) concluded that the increase in P-type flies in a population is unlikely to result from positive selection for P element-carrying chromosomes. Similarly in these experiments there was little evidence of selection for P chromosomes as the frequency of white eyed males decreased slowly each generation after generation 4. This does not completely eliminate the posibility of this type of selection since the white eye marker is at the tip of the X chromosome. To be able to eliminate this possibility, each chromosome would have to have multiple markers.

The number of P elements within a particular generation was measured by dot blot hybridization. These results demonstrate that the increase in P element copy number within a particular generation as measured by DNA hybridization is matched by an equivalent increase in ability to induce gonadal dysgenesis.

This indicates two things. First it supports the work of Kidwell et al. (1981) and Kiyasu and Kidwell (1985) in that the increase they found in P-type flies probably results from an increase in P elements rather than through chromosomal segregation. Second, the simplest explanation for this rapid increase in P element copy number is by replicative transposition of the P elements. The other possible explanation would be that P elements are disproportionately represented in the offspring of P-M crosses, analogous to what happens with segregation distortion. Thus these results provide further evidence supporting the idea that P elements can undergo replicative transposition. The use of the HIII P element fragment did not allow distinguishing between an increase in P factors and an increase in P elements. However, the 1:1 relationship between P element copy number and P element activity (Figure 2.4) would suggest that the ratio of P factors to P elements had not changed significantly from generation 0 through 20.

In summary, these results support previous work which has shown an increase in P-type flies in mixed P-M populations (Kidwell et al., 1981; Kiyasu and Kidwell, 1984; Kidwell, 1986). It had been suggested in Chapter 1 that the apparent rapid spread of P elements in population cages might result more from chromosomal segregation and recombination than any actual increase in the copy number. However, the DNA hybridization data indicate that gonadal dysgenesis is probably a fairly accurate measure of the proportionate number of P elements within a

particular strain. Thus P elements seem to be able to increase rapidly in frequency in a mixed population presumably as a result of replicative transposition.

CHAPTER 4

MATING EFFECTS AND FURTHER DILUTION EXPERIMENTS IN MIXED P-M POPULATIONS

Introduction:

In the experiments described in Chapter 2, repeated backcrossing of a P-type strain to an M-type strain resulted in a reduction in the number of P elements in each subsequent generation. In contrast, in the experiments described in Chapter 3, low frequencies of P-type flies were placed in population cages with M-type flies and allowed to mate randomly for 20 generations. In these experiments P elements were found to spread rapidly through the population such that by generation 20, 60% to 100% of the flies were P-type. This rapid increase in the percentage of P-type flies was suggested to have resulted from replicative transposition of the P elements.

The experiments outlined in Chapters 2 and 3 were designed to model the introduction of a few migrant P flies into a large population of M flies. However, there are two ways in which these experimental designs clearly differ. First, the backcross experimental design results in very strong selection against the P element. P-type chromosomes will be diluted 50% each generation in these crosses and if there is selection against the F1 offspring of a particular generation, selection will be greater than 50%. In contrast, depending on the breeding combination in the randomly mating population, P elements might not be diluted to this extent and may be able to maintain their

copy number at higher levels. Thus the Chapter 2 experiments are equivalent to introducing a P fly into an infinitely large M population, whereas the population cage experiments are equivalent to introducing a P fly into a finite population.

Second, in the experimental design used in Chapter 2 the breeding scheme is completely specified, whereas in the population cage studies the flies will breed randomly. One factor that might have a large effect on the ability of P elements to transpose is the particular mating combination a fly resulted from. This could operate in two fashions. First, due to the differential segregation of X chromosomes, male and female offspring will get differing numbers of P elements and therefore different rate of transposition might result. Second, in the case of cytotype, it is known that parental genotypes and ancestral genotypes are extremely important in determining the cytotype of a fly (Engels, 1979; Kidwell, 1984). This appears to result from the differential inheritance of the regulator that determines cytotype. If a similar phenomenon were to affect the amount of transposase that was being produced by a fly, there might be differential rates of P element activity depending on the genotypes of a fly's ancestors.

The experiments outlined in this Chapter were designed to further elucidate the differences between Chapters 2 and 3. The first set of experiments were designed to more accurately measure what the effect of parental genotype would be on the P element activity of a particular fly. This was modelled by setting up

breeding experiments in which the P element activity and cytotype were measured for all possible mating combinations up until generation 4. The second set of experiments are very similar to the experiments detailed in Chapter 3. Population cages were initiated with 10% P-type flies, as outlined in Chapter 3, but each generation the population was mixed with an equal number of M-type flies. This is the equivalent of diluting the population cage flies by 50% each generation with M-type flies.

Given previous work, it is clear that there are conditions under which P elements can increase rapidly in frequency, and other conditions where they can be rapidly lost from a population. With the experiments outlined in this Chapter, I hoped to be able to clearly understand what conditions lead to the most rapid rates of transposition for P elements.

Materials and Methods

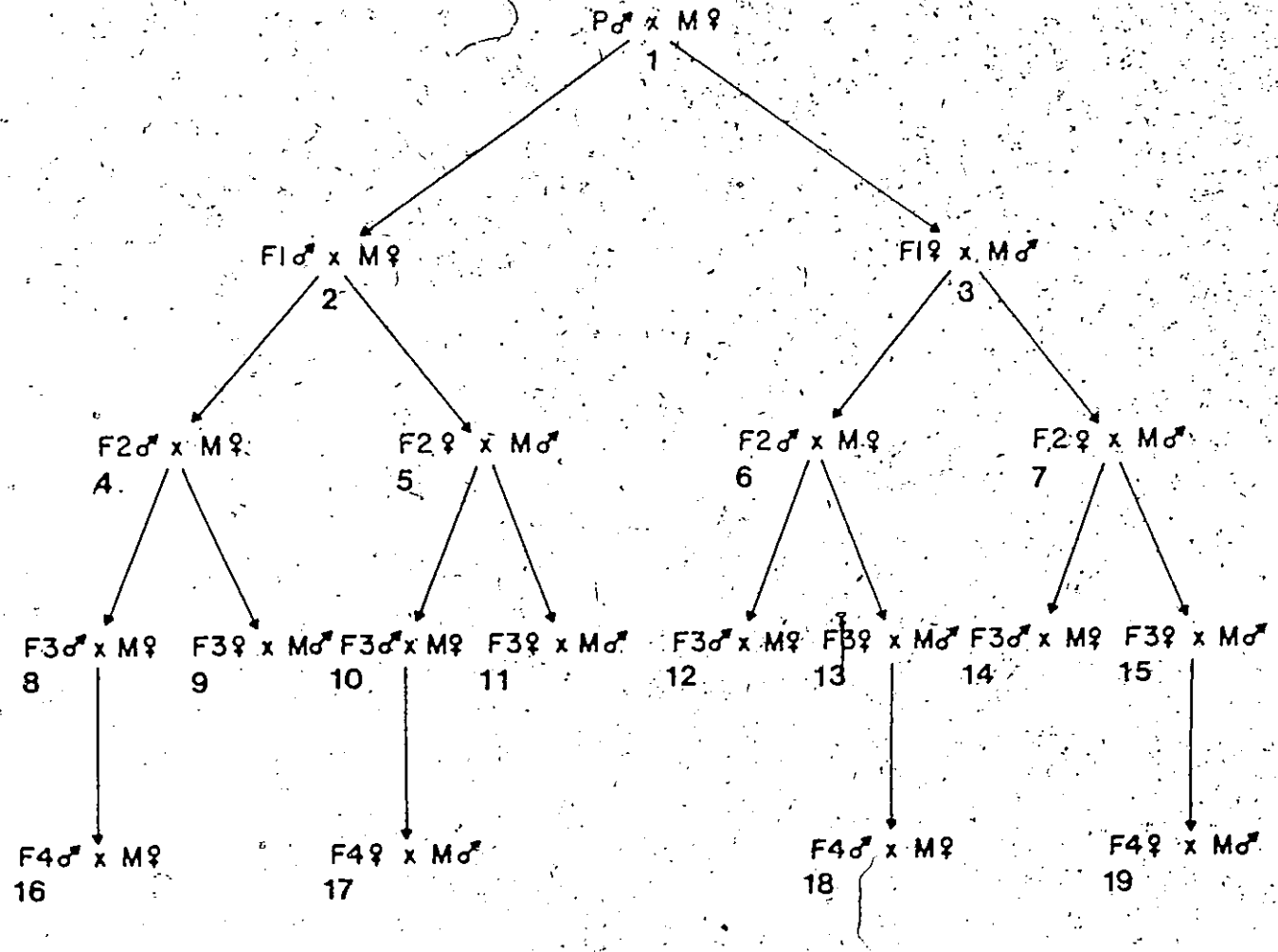
Stocks:

(1) Harwich, (2) π_2, (3) Canton-S. See Chapter 3 for detailed descriptions of these stocks.

Backcross Experiments: The experimental design used in these experiments is outlined in Figure 4.1. Each particular cross is identified by an number, placed just below the cross. The initial cross of a Harwich (P) male X Canton S (M) female is potentially dysgenic, but after that the number of P elements in each successive generation should decrease as a result of dilution. The major difference between this experimental design and that of Chapter 2 is that the Cross A of Chapter 2 covers only one of the possible crosses outlined in Figure 4.1 (Crosses 1, 2, 4, 8, 16) whereas in a randomly breeding population there would be a rapid increase in the number of potential crosses each generation. All flies were maintained at 21°C in standard (95mm Ht x 25mm Dm) shell vials and raised on Carolina Instant Drosophila media. Each cross was started with approximately 30 flies and each generation the flies were screened for P element activity and P cytotype. DNA was extracted from 100 to 200 flies and the P element copy number calculated using DNA hybridization.

Population Cages: All population cage experiments were carried out at 21°C and raised on Yeast Agar media (900 mls H_2O, 100 gms sugar, 50 gms dried yeast, 15 gms agar). All other conditions were as described in Chapter 3. Two different experiments were initiated with 10 % P-type flies by initially

FIGURE 4.1: Outline of the mating scheme used in the backcross experiments. In order to simplify discussion, each cross is identified by a number below the cross. For example, the F2♂ x M♀ cross whose parents were F1♀ x M♂ is cross 6.


P♂ x M♀
1
F1♂ x M♀
2
F1♀ x M♂
3
F2♂ x M♀
4
F2♀ x M♂
5
F2♂ x M♀
6
F2♀ x M♂
7
F3♂ x M♀
8
F3♀ x M♂
9
F3♂ x M♀
10
F3♀ x M♂
11
F3♂ x M♀
12
F3♀ x M♂
13
F3♂ x M♀
14
F3♀ x M♂
15
F4♂ x M♀
16
F4♀ x M♂
17
F4♂ x M♀
18
F4♀ x M♂
19

mixing 20 Harwich flies (Experiment A) or 20 π_2 flies (Experiment B) each with 180 Canton-S (M-type) flies. Four replicate populations were set up for each experiment. In all experimental populations, the flies were diluted each generation with 50% M-type (Canton-S) flies, by mixing 200 M-type flies with 200 flies from each cage. These dilutions were carried out every generation up to generation 8 when they were stopped. Subsequent generations of these populations were transferred without dilution as discrete generations to new population cages.

Since the dilution experiments were started with fairly low frequencies of P-type flies (10%), subsequent experiments were carried out to test the effect 50% dilution would have on populations with high P element copy numbers. In order to do this two replicate cages were chosen from populations A and B described in Chapter 3 and diluted 50% each generation, from generation 20 through generation 25. By generation 20, these populations were at least 60% P-type, as measured by P element activity and P element copy number, and had been shown to be able to increase in P element frequency very rapidly. The other two replicates were maintained as controls through generation 25. P element activity and P cytotype had been previously measured in generations 5, 10, 20 (Chapter 3) and were remeasured in generation 25. Likewise in generation 25, flies were frozen and their DNA extracted to measure P element copy number.

Method of P element activity determination: Individual males were mated with 3-4 Canton-S females, the F1 hybrid females were

aged 2-4 days and their ovaries dissected according to the methods of Schaefer et al. (1979). Ten females from each individual mating were scored for sterility and the percentage dysgenesis was calculated as the mean of 5 individual matings.

Method of Cytotype determination: Individual females were mated with 2-3 Harwich males, the F1 hybrid females were aged 2-4 days and their ovaries dissected (Schaefer et al. 1979). Ten females from each individual mating were scored for sterility and the percentage dysgenesis calculated as the mean of 5 individual matings.

Statistical Analysis: Changes in P element activity were analysed using analysis of variance (ANOVA), and Duncan's multiple range tests as outlined in Chapter 2. For the backcross experiments, since analysis of variance does not react favourably to empty cells, the F3 crosses 9, 11, 12 and 14 were eliminate from the analysis, thereby producing three generations, four matings and 3 replicate populations within each mating.

Detection of P elements in crosses by hybridization: The presence of P element sequences in a particular generation was investigated by DNA hybridization. DNA was extracted from 100-500 flies and dot blots and P element copy number calculations performed as outlined in Chapter 3.

Results

Backcross Experiments: The evolution of P element activity in each of the different mating possibilities is shown in Figure 4.2. The level of P element activity was initially very high in the F1 generation and then decreased with each subsequent backcross generation. However there were significant differences in the amount of P element activity in the different backcross matings. Males resulting from the F1♀ x M♂ mating had a much higher P element activity than did those of the F1♂ x M ♀ mating. The progeny from the F1♀ x M♀ mating continued to produce males with higher P element activity in the F2 and F3 generations. Changes in P element activity for each mating were analysed using analysis of variance (ANOVA). As illustrated in Table 4.1 the main effects, generation and mating were highly significant as was the generation X mating effect. All other effects were not significant. Differences between generations were analysed using a Duncan's multiple range test (Table 4.2). Generations 2 and 3 were found to differ significantly from each other however, there was no significant difference between generations 3 and 4 in P element activity.

The cytotype of each different backcross mating was monitored each generation. In contrast to P element activity all matings had almost 100% M cytotype. The cytotype of the F1♀ from the initial P♂ X M♀ cross was 97.8% M-type, but subsequent generations all had greater than 98% dysgenic Fis's, indicating a completely M cytotype. Due to the low level of variance in these

FIGURE 4.2. Changes in P element activity with each backcross mating. The percentage P element activity decreases with each generation, but differs significantly depending on the mating. P element activity was calculated as described in the text.

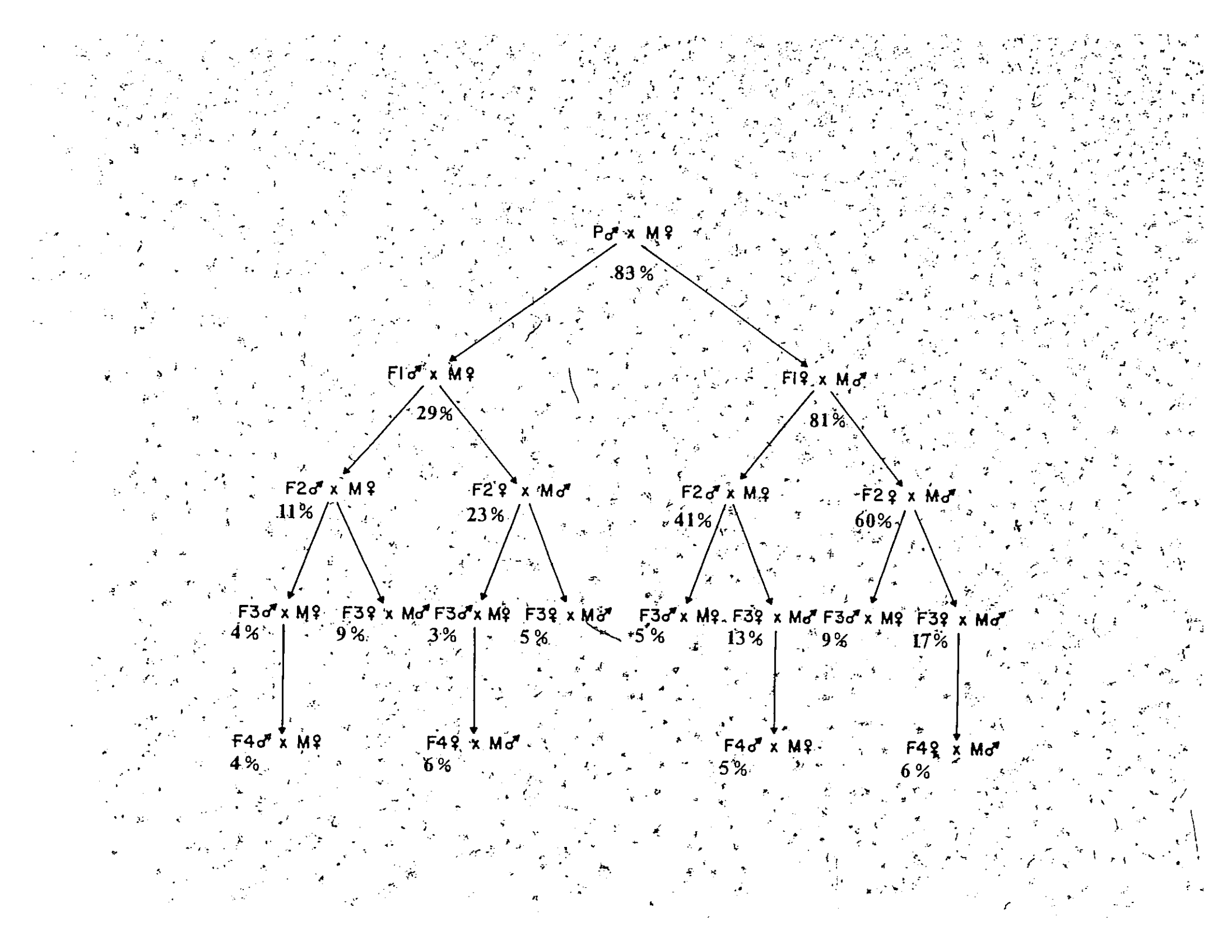

P♂ x M♀
83%
F1♂ x M♀
29%
F1♀ x M♂
81%
F2♂ x M♀
11%
F2♀ x M♂
23%
F2♂ x M♀
41%
F2♀ x M♂
60%
F3♂ x M♀
4%
F3♀ x M♂
9%
F3♂ x M♀
3%
F3♀ x M♂
5%
F3♂ x M♀
5%
F3♀ x M♂
13%
F3♂ x M♀
9%
F3♀ x M♂
17%
F4♂ x M♀
4%
F4♀ x M♂
6%
F4♂ x M♀
5%
F4♀ x M♂
6%

TABLE 4.1: Three way analysis of variance of P element activity in the backcross experiments.

TREATMENT	D.F.	SUM OF SQUARES	F. VALUE
Generation (A)	2	284.3	71.3 ***
Mating (B)	3	122.3	20.4 ***
Population (C)	2	1.6	0.4 N.S.
A X B Interaction	6	108.6	9.1 ***
B X C Interaction	4	11.7	1.5 N.S.
A X C Interaction	6	3.4	0.3 N.S.
A X B X C Interaction	12	23.7	1.0 N.S.
Error	144	843.0	

*** $p < 0.001$

D.F. = degrees of freedom

N.S. = Not significant

TABLE 4.2 Duncan's multiple range test of P element activity for generation effect.

Generation

Generation	Mean	Grouping*
2	3.37	A
3	0.93	B
4	0.52	B

* Means with the same letter are not significantly different

populations, analysis of variance was not performed on the data.

There are two possible explanations for the differences in P element activity in the F2 and F3 generations. First, the ability of a male fly to induce dysgenesis may in part be determined by the genotype of the parents of that fly. Second, due to differential segregation of the X chromosome, males from the different matings could have different numbers of P elements in their genome. This second possibility was investigated in two ways. First, the DNA was extracted from F3 flies and the percentage of P elements calculated using DNA hybridization. Second, by making several assumptions, the average number of P elements in each backcross mating up to generation 3 was calculated assuming first no transposition and then 80% transposition per generation. The results from these two analyses are shown in Table 4.3 and 4.4. From a theoretical standpoint the number of P elements in the offspring from the F1s x M- mating have slightly higher levels of P elements, since they retain some of the P X chromosome. However, assuming no transposition has occurred, there should be no difference in the P element copy number between the different F2 and F3 matings, other than the distinction between offspring resulting from cross 2 versus cross 3. Using DNA hybridization, it can be seen that there is very little difference between the number of P elements in each of the four F3 populations. Thus the amount of P element activity in the F3 generation flies does not seem to be closely correlated to the number of P elements found in each experimental

TABLE 4.3: Percentage P element sequences compared to Harwich using DNA hybridization, for backcross mating experiments. See Figure 4.1 for matings.

	Experimental Mating							
	8		10		12		14	
	A	B	A	B	A	B	A	B
Percentage P elements	7.9%	8.5%	8.0%	7.4%	13.7%	11.4%	13.1%	14.2%

Note: A and B are replicate populations from the separate matings.

TABLE 4.4: Percentage P element sequences compared to Harwich based on theoretical calculations*, in each generation for backcross mating experiments. The 80% Trans. refers to the % P elements if there had been 80% transposition per generation. See Figure 4.1 for matings.

	Backcross Mating			
Generation 1		2	3	
% P elements 80% Trans.		38.5% (69.3%)	50% (90%)	
Generation 2	4	5	6	7
% P elements 80% Trans.	19.3% (62.4%)	19.3% (62.4%)	25% (81%)	25% (81%)
Generation 3	8	10	12	14
% P elements 80% Trans.	9.6% (56.1%)	9.6% (56.1%)	12.5% (72%)	15.4% (72%)

* The theoretical calculations are based on the following assumptions. (1) No transposition occurs, (2) there is no selection, (3) each chromosome carries a percentage of P elements equal to the percentage of total DNA in that chromosome. The chromosomal percentages for a female fly of total DNA are (X = 23.0%), (2 = 37.6%), (3 = 38.3%), (4 = 1.2%) (Lindsey and Grell, 1968).

population.

Population Cage Experiments: The change in P element activity and cytotype in the first set of population cage experiments is demonstrated in Figures 4.3 and 4.4 respectively. Appendix 4.1 provides a more detailed description of this data. In both experiments P elements were rapidly lost from the population such that by generation 5 there was very little P element activity. In addition, all populations had 100% M cytotype by generation 5 and retained their M cytotype through to generation 20. Analysis of variance of P element activity clearly demonstrates that most of the variation in the population was random (Table 4.5). First, the model only accounts for 39.1% of the total variation and second the main effect of population (which represents the variation between replicate cages) was the most significant effect. These results are not suprising however, given the almost complete absence of P element activity in the experimental populations.

The measurement of P element copy number demonstrates that P elements have been almost totally lost from the experimental populations (Table 4.6). Appendix 4.2 provides the more detailed data used in Table 4.6. By generation 2 the mean copy number had been reduced to 4.0% or below, while subsequent generations had less than 0.5% P element sequences. The exception to this is replicate B of the Harwich experiment: This population had low, but detectable levels of P elements in generations 5 and 10.

FIGURE 4.3: Changes in P element activity at Generations 5, 10 and 20 for the experimental population cages.

The experimental starting freqencies are,

A = 10% Harwich X 90% Canton-S

B = 10% π_2 X 90% Canton-S

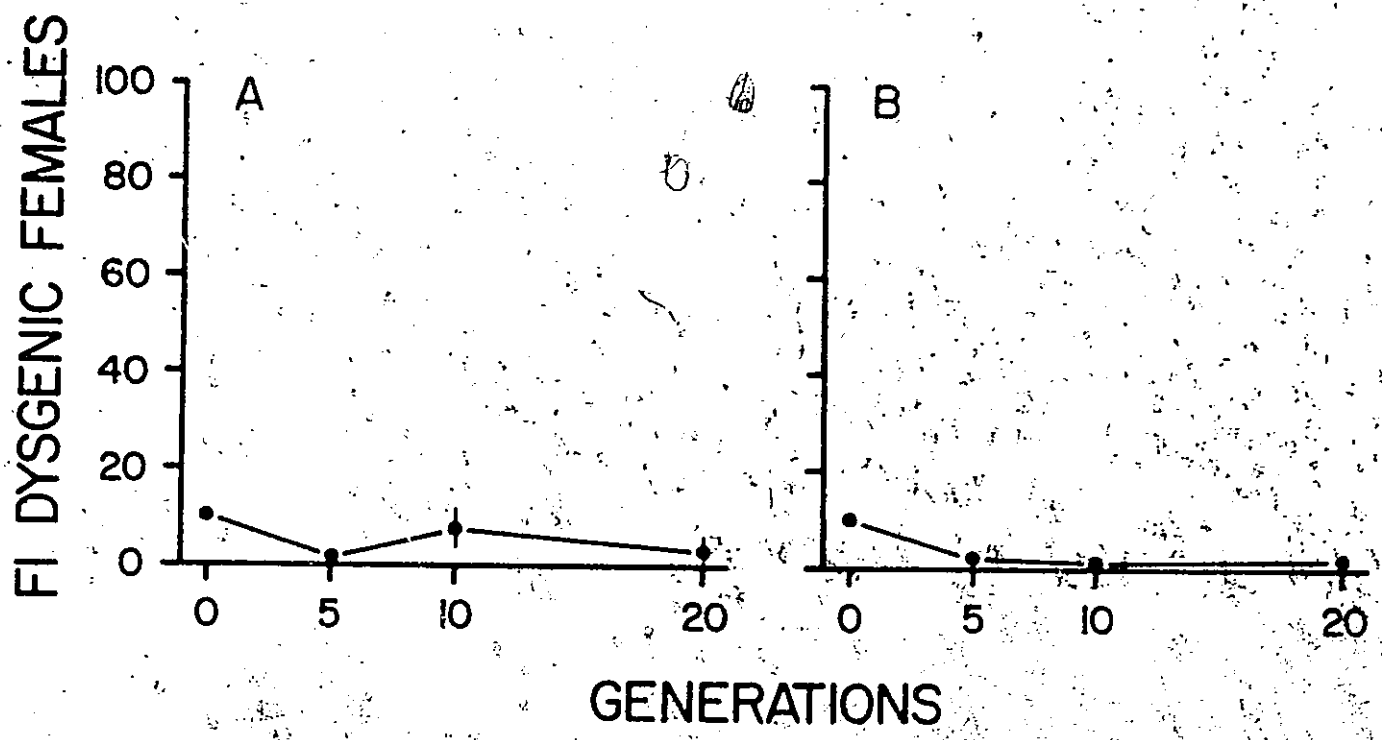

F1 DYSGENIC FEMALES
100
80
60
40
20
0
A
B
0
5
10
20
0
5
10
20
GENERATIONS

FIGURE 4.4: Changes in cytotype at Generations 5, 10 and 20 for the experimental population cages.

The experimental starting freqencies are,

A = 10% Harwich X 90% Canton-S

B = 10% π_2 X 90% Canton-S

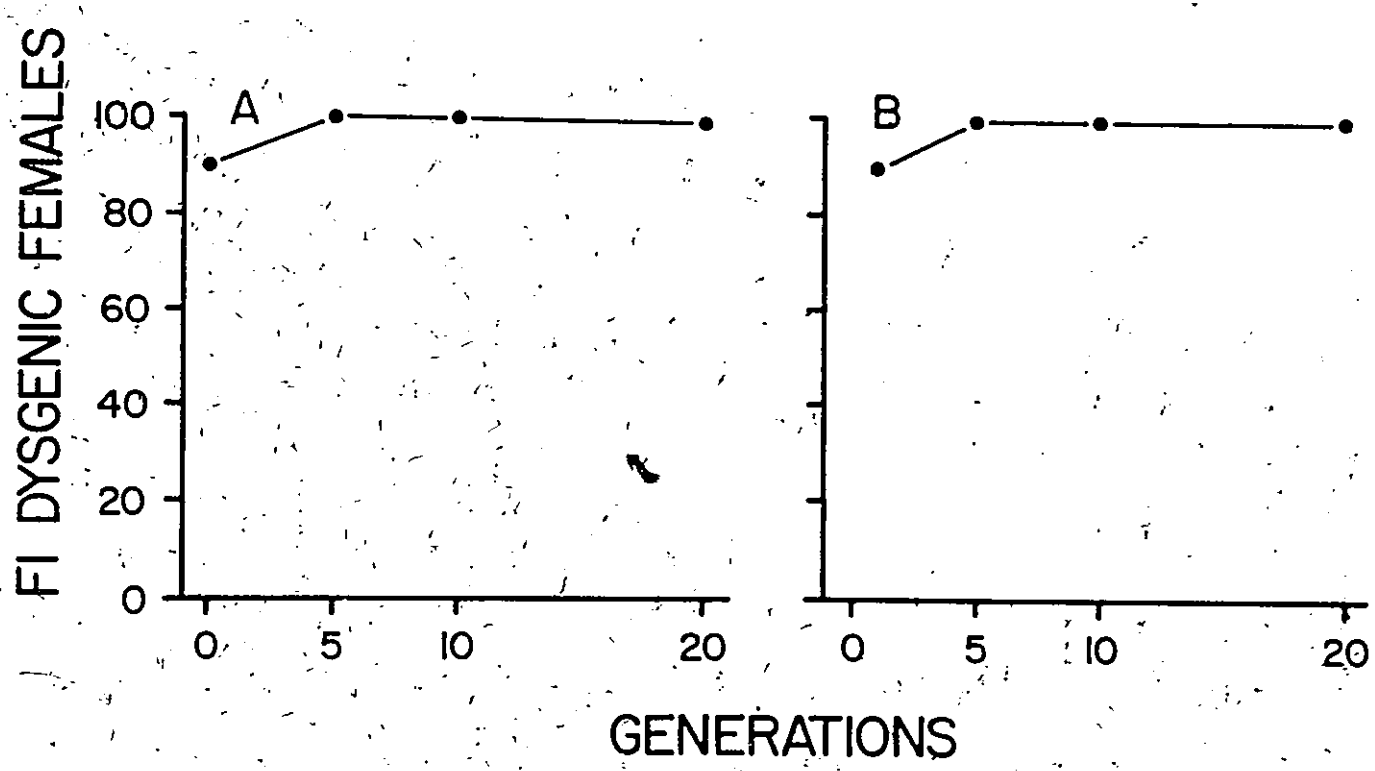

F1 DYSGENIC FEMALES
100
80
60
40
20
0
A
B
0
5
10
20
0
5
10
20
GENERATIONS

TABLE 4.5: Three way analysis of variance of P element activity in the population cage experiments.

TREATMENT	D.F.	SUM OF SQUARES	F VALUE
Generation (A)	2	4.0	2.7 *
Experiment (B)	1	4.4	5.8 **
Population (C)	3	10.4	4.6 ***
A X B Interaction	2	7.2	1.8 **
B X C Interaction	6	7.6	1.7 N.S.
A X C Interaction	3	5.3	2.4 *
A X B X C Interaction	6	7.5	1.7 N.S.
Error	96	72.4	

***=p $\leq$ 0.01, **=0.05 $\leq$ p $\leq$ 0.01, *=0.1 $\leq$ p $\leq$ 0.05

D.F. = degrees of freedom

N.S. = Not significant

TABLE 4.6: Percentage P element sequences compared to Harwich using DNA hybridization, for population cage experiments. Samples 1 and 2 are replicate populations from the same experiment.

Generation	Population		Starting Frequency 1%	10% Dilution
1	Harwich	1	3.5%	0.7%
		2	2.5%	4.0%
2	π_2	1	9.2%	0
		2	7.1%	0.2%
	Harwich	1	27.9%	0
		2	30.1%	0.1%
5	π_2	1	20.3%	0
		2	29.2%	0
	Harwich	1	25.1%	0
		2	31.1%	0.5%
10	π_2	1	53.4%	0
		2	59.1%	0
	Harwich	1	59.4%	0
		2	60.7%	13.1%
20	π_2	1	89.8%	0
		2	86.0%	0
	Harwich	1	0	
25		2	0	
	π_2	1	22.0%	Dilution through Generation 25
		2	25.3%	
	Harwich	1	51.3%	
25		2	31.6%	
	π_2	1	82.0%	Control; No dilution
		2	79.3%	

However, by generation 20, the amount of P element sequences had increased to 13.1%. Since these experiments were not diluted beyond generation 8, this indicates a relatively rapid rate of increase of P element sequences as a result of replicative transposition. This can be more clearly seen in Appendix 3.2 which shows a clear DNA signal from generation 20.

As in Chapter 3, the possibility that there could be positive or negative selection for P elements because of selection for P element carrying chromosomes was monitored by taking advantage of the fact that the Harwich strain used had a white eye colour. Therefore the frequency of the white-eyed males in any generation will reflect the frequency of Harwich X chromosomes in the previous generation. Table 4.7 illustrates the white-eyed males in each generation from 1 through 10. The frequency of white eyed males in these experiments decreased approximately 50% each generation, suggesting that little or no selection was occurring.

The results from these first set of population cage experiments confirm the results in Chapter 2, that P elements can be lost from a population by dilution. However, since the initial frequencies in these two experiments were fairly low (10%), it could be argued that dilution would not have the same effect in a randomly mating population if the percentage of P elements was initially much higher. In order to test this, two replicates from populations A and B of Chapter 3 were diluted from generations 20 through 25 and then tested for P element

TABLE 4.7: Percentage of white eyed males in each generation during population cage experiments (X + S.E., n =4).

Generation	Population A	Theoretical*
Initial %	10%	10%
1	9.9% + 1.1	10%
2	5.3% + 1.1	5%
3	2.6% + 0.6	2.5%
4	0.9% + 0.4	1.2%
5	0.2% + 0.1	0.6%
6	0% + 0	0.3%
7	0.1% + 0.1	0.2%
8	0% + 0	0.1%
9	0% + 0	0%
10	0% + 0	

* Assumes no selection.

activity and cytotype. The results from these experiments are outlined in Table 4.8. While the frequency of P elements was fairly high in generation 20, 5 generations of dilution substantially reduced the P element activity and cytotype (Table 4.8). However, the 2 strain seems to have more P element activity than Harwich, as measured by both P element activity and P element copy number. DNA analysis indicated that the reduction in P element activity was matched by a subsequent reduction in P element copy number. These results are shown in Table 4.6. While the control lines retained high copy numbers of P elements, the dilution experiments had very reduced levels, from 0% to 25% of the Harwich control strain.

TABLE 4.8: Changes in P element activity and cytotype in Populations A and B from generations 5 through 25. From generations 1 through 20, flies were transferred each generation, (Chapter 2) whereas in generation 20 through 25 two of the replicates from each populations experiments were diluted with 50% M-type flies each generation.

P element activity	Gen	Pop. A (Harwich)	Pop. B (π_2)
	5	21.2%	30.5%
	10	43.5%	72.0%
	20	65.0%	92.0%
	25 Dilution	13.0%	17.0%
	25 Control	90.0%	99.0%

P cytotype	Gen	Pop. A (Harwich)	Pop. B (π_2)
	5	98.5%	91.7%
	10	100%	71.7%
	20	12.0%	6.0%
	25 Dilution	99.0%	97.0%
	25 Control	8.0%	7.0%

Discussion

Breeding Experiments: The results of these experiments strongly suggest that the level of P element activity in a particular male fly is determined in part by the genotypic ancestry of that fly. In general, it seems that males have a higher level of P element activity when their X chromosome has been passed down through a female line.

There is evidence in the literature to support this idea. Colgan and Sved (1982) found that the males resulting from a F1♀ x M♂ had 84% P element activity versus the 81% observed above (Cross 3). In contrast, males from a F1♂ x M♀ had 42% P element activity versus the 29% shown in Figure 4.2 (Cross 2). Kidwell (1983a) found that the levels of P element activity were higher when the X chromosome had had an opportunity to be contaminated with P elements in both the F1 and F2 generations. Kidwell (1983a) also found that rate of contamination of the X chromosome was significantly higher than that of the autosomes. However, because the autosomes she used were balancers, with multiple inversions, it is possible that these produced results not typical for homosequential autosomes.

There are several ways to explain this ancestral genotype effect. First, although Kidwell (1983a) found that the X chromosome had the highest levels of P element activity of all chromosomes, the differences we see in P element activity between the different matings cannot be explained solely by the effect of the X chromosome. For example, males resulting from cross 6 have

an M X chromosome, as do males resulting from cross 4 and 5, yet there are significant differences in the males ability to induce gonadal dysgenesis. However, although the X chromosome may be M-type, the males from each different cross may have different numbers of P elements in their genome, due to different rates of transposition in the F1 and F2 generations. If this were the case, one would expect to see these differences using DNA hybridization. However, the DNA hybridization data correspond very closely to what one would predict on the basis of the dilution of P elements with no transposition. The preferential increase in the number of P factors however, might cause substantial differentes in P element activity, without showing up as large differences in P element copy number. Thus this is one case where the use of different probes might provide interesting results.

The alternative explanation to differences in P factor or P element copy number is that P elements in the males from crosses 12 and 14 are much more active than in crosses 8 and 10, and are therefore capable of producing much higher levels of dysgenesis. Unfortunately there is no simple physical model to explain how this might happen. One could argue that somehow the level of transposase in crosses 12 and 14 is much higher than in crosses 8 and 10, or alternatively it could be argued that the small differences in number of P elements was causing drastic differences in the level of gonadal dysgenesis. However, the general view of P element induced gonadal dysgenesis is that when

the male sperm enters the egg, the level of activity of the male genome will be determined by the female egg. This view makes it difficult to understand how the ancestral genotype effect might work. It seems clear that to really understand this effect would require a number of carefully designed experiments. My personal feeling is that one should first test whether there are different numbers of P elements in the different crosses by using marked chromosomes and insitu hybridization. These two techniques would allow one to follow several individual elements and their ability to induce gonadal dysgenesis. Then one could use different molecular probes to look at whether you are seeing differential increases in P factors over P elements.

Although there was a rapid decrease in P element activity with each generation of backcrossing, the cytotype remained almost 100% M-type during these experiments. It has been clearly shown that the progeny of a P x Ms will have an M-cytotype and this will then change slowly to P-type (Engels, 1979). However, in these experiments, each backcross was to a M-type fly, so the P cytotype was never really given a chance to establish itself. One would then predict, based on previous work (Chapter 3, Kidwell et al., 1981; Kidwell, 1986) that it would take quite some time for a P cytotype to be established, and until then P elements could transpose freely.

Population Cage Experiments:

The population cage experiments provide strong support for

the experiments described in Chapter 2. It appears that P elements can be lost from experimental populations if there is a strong dilution effect. This loss of elements occurs both when the starting frequency of P elements in the population cages is low and when it is fairly high. Thus there does not seem to be a certain threshold frequency of P elements above which they are able to maintain their copy number at a relatively high level. Moreover, there was no evidence of selection for P chromosomes as the frequency of white-eyed males decreased approximately 50% each generation. An interesting contrast to this rapid loss of P elements was population 2 of the Harwich dilution experiments. This population had extremely low levels of P elements in generation 10, after it had gone through 8 generations of dilution. However, by generation 20, it had rebounded to approximately 13% of the wild type levels of P elements. Therefore, as had been seen in Chapter 3, under conditions where there is no dilution there are examples where even though the level of P elements is extremely low, they are capable of increasing in frequency fairly rapidly.

In summary, the differences between the experiments in Chapters 2 and 3 seem to result from two factors. First, 50% dilution is a very strong selective force, and although P elements can transpose quite rapidly, they are not able to overcome this level of selection. This data might suggest that if P elements were to invade natural populations, they would be more likely to spread in smaller populations where continual backcrossing is

less likely to occur. However, there are also distinct differences in levels of transposition that occur as a result of the ancestral genotype of a fly. This is similar to what has been described for the regulation of cytotype (Engels, 1979; Kidwell, 1985); however unlike cytotype no physical model has been developed to explain these results.

CHAPTER 5

POPULATION GENETICS MODELS FOR THE EVOLUTION OF TRANSPOSABLE ELEMENTS

Introduction:

As the ubiquity of transposable elements has become apparent, there has been a growth in the theoretical literature on the evolutionary biology and population genetics of these elements. Much of this literature is concerned with explaining the main features of the distribution and abundance of transposable elements within a host's genome and in a population, as functions of variables such as transposition and excision rates (Ohta, 1981, 1983; Charlesworth and Charlesworth, 1983; Langley et al., 1983; Kaplan and Brookfield, 1983; Kaplan et al., 1985). These models will not be discussed, since they address a very different question than does this thesis. Other authors have developed models to explain the spread of transposable elements in sexually reproducing organisms (Hickey, 1982; Ginzberg et al.,1984), while several authors have begun to look at the evolution of self-regulated transposition (Doolittle et al., 1984; Charlesworth and Langley, 1986).

In this chapter I will discuss (1) models of the spread of transposable elements and (2) the self-regulation of transposable elements. The models of the spread of transposable elements ask the question, "How can the number of genomes containing a TE (transposable element) increase in frequency over a period of time in a natural population"? The models of self-regulated

transposition deal with how transposable elements could evolve the ability to self-regulate. P-M hybrid dysgenesis has been an important system in helping to develop both of these types of models.

The Spread of Tnansposable Elements:

The evolutionary origin and maintenance of prokaryotic TE's represents a slightly different problem than in eukaryotic organisms. Selfish genes (eg: transposable elements) cannot spread in a deterministic fashion among asexual organisms with strictly clonal patterns of reproduction. This is because although the TE can increase in certain genomes they cannot colonize new genomes (Cavalier-Smith, 1980). Their evolutionary fate is dependant on the survival of their host genomes, and if they confer no selective advantage on these host genomes, there will be no systematic tendency towards an increase in the frequencies of genomes containing the element (Hickey, 1982).

In contrast to prokaryotic models, in diploid obligate sexual organisms, recombination results in a transposable element spreading deterministically in a population even though the element may have serious deleterious effects on its host organism. Hickey (1982) developed a model to explain the spread of mobile genetic elements in diploid obligately sexual organisms. He points out that the rate of increase of a TE will be determined by (1) the frequency of the TE in the population and (2) the selection acting on those host organisms carrying the

element. The average copy number per cell of a TE in a population may be expressed as:

$$f = \frac{a \times b}{N}$$

where a is the number of copies per genome, among those genomes which contain the TE; b is the number of genomes containing at least one copy of the element and N is the total number of genomes in the population. Therefore in any given population, the value of f can increase due to an increase in the value of a or b. However, in the event that a increases and b does not increase, the element cannot be said to have spread in the population; its overall frequency increases, but this is because those genomes that already contains some copies acquire more copies. This model makes several important conclusions. First, it points out that a TE can spread in natural populations even though that element is actually harmful to its host. The maximum value of the selection coefficient that would still permit the spread of a transposable element is shown in Table 5.1. The important point to be noted is that if the transposable element is passed on in all of the gametes of an organism, it can reduce the fitness of its host up to 50% and still spread rapidly in the population. Second, once the element reaches an appreciable frequency in the population, the selection against host individuals can be much greater without impeding the elements continuing spread to fixation.

TABLE 5.1: Relationship between the frequency of a transposable element and the maximum level of negative organismic selection which allows for its continued spread (After; Hickey, 1982; Table 4).

Frequency of element (p)	Maximum value of selection coefficient (s)
0	0.50
0.1	0.53
0.2	0.55
0.5	0.67
0.8	0.83
0.99	0.99

The model also predicts that those classes of organisms in which biparental reproduction is rare or absent would be incumbered by less nonfunctional or parasitic DNA than most vertebrates. Finally, according to this model, the spread of a TE depends on sexual reproduction. Therefore, any TE that caused sex in its host would favour its own spread. Thus the intriguing possibility exists that sex itself is a product of parasitic genes (Hickey, 1982).

The model of Ginzberg et al. (1984) is very similar to that of Hickey (1982). They developed a simple model of the spread of a TE in a large sexually reproducing, panmictic population and found that a transposable element will invade a population even if it reduces the fitness of hybrid individuals carrying it, provided that the fitness loss is not greater that a certain critical value" (Ginzberg et al., 1984, p. 331). This critical value was in turn determined by the "infectivity" or transposition rate of the transposon. As mentioned earlier for prokaryotes, the transposition rate, or infectivity of a transposon will affect conditions under which the element will spread. As Ginzberg et al. (1984) point out , in order for the transposable element to spread:

$$B < \frac{S}{1 - S}$$

where B is the infectivity of the transposon and s is the selection coefficient. Ginzberg et al., (1984) developed their model to specifically mimic the P-M and I-R systems and found

that given the parameters, s=0.1 (10% selection against the hybrids) and B=0.9 (90% infectivity) P and I elements had gone from an initial frequency of 10^{-6} to fixation in about 65 generations. More interestingly, they found that it took approximately 21 generations for the frequency of P elements to increase from 1% to 90% and 16 generations to increase from 10% to 90%. Figure 5.1 shows the spread of transposable elements through a population as modeled by Ginzberg et al. (1984). The process of spread is practically invisible for approximately half of the time period, while the visible portion of the curve (1% to 99%) appears very similar for a variety of combinations of parameters (Ginzberg et al., 1984).

How does this model compare to the population cage experiments described in Chapter 3. Table 5.2 compares the rate of spread of the P element based on the model of Ginzberg et al. (1984) with the rate of spread of P elements in the population cages using either P element activity or P element copy number. In general, the results from the population cages are closely matched by the theoretical predictions. The major difference seems to be in the early generations (generations 0 to 10) where there is a much faster rate of spread in the cages than predicted by the model. This could be due to selection for the P element carrying chromosomes as suggested by Table 3.7. Alternatively, this model may not accurately describe P element spread in the P-M system, or the parameters used by Ginzberg et al. (1984) may differ from what is actually happening in the population cages.

FIGURE 5.1: Typical curves for the spread of transposable elements through a population. Two curves are shown for the same values of parameters (s = 0.1 and B = 0.9, See text). This Figure is from Ginzberg et al. (1984), Figure 2.

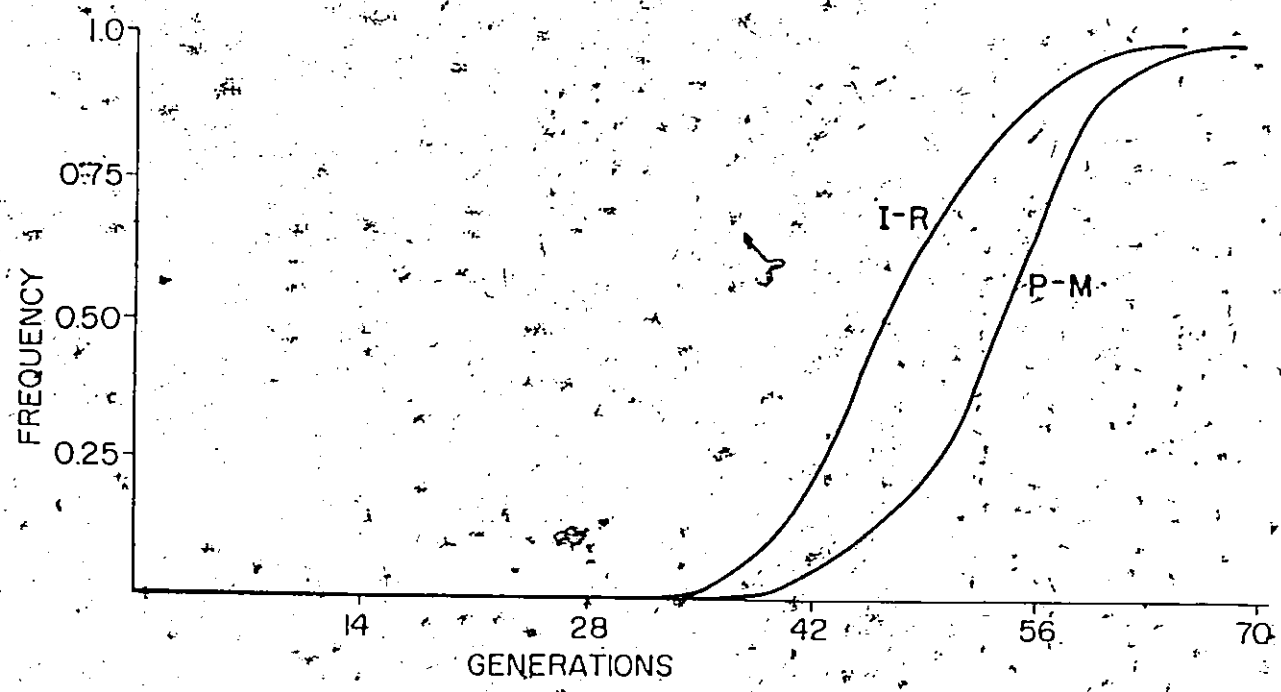

1.0
0.75
0.50
0.25
FREQUENCY
I-R
P-M
14
28
42
56
70
GENERATIONS

TABLE 5.2: Comparison of the rate of spread of P elements in a population, based on the theoretical model of Ginzberg et al. (1984) versus the population cages studied in Chapter 3.

A. 1%	Theoretical values	P element activity Harwich		P element copy # Harwich	
Generation					
0	1%	1%	1%	1%	1%
5	9%	21%	30%	29%	25%
10	22%	44%	72%	28%	56%
15	49%	–	–	–	–
20	85%	65%	92%	60%	88%

B. 10%	Theoretical values	P element activity Harwich		P element copy # Harwich	
Generation					
0	10%	10%	10%	10%	10%
5	25%	48%	38%	15%	19%
10	57%	81%	42%	70%	42%
15	89%	–	–	–	–
20	99%	97%	65%	73%	78%

Ginzberg et al's. (1984) model does not accurately describe the P-M system in two ways. First, cytotype has not been incorporated into the model. Therefore in the later generations (generations 10 to 20), as the population slowly switches cytotype there will be a decrease in the rate of spread of the element. This can be seen in part in Table 5.2 in that the rate of spread from generations 10 through 20 for either starting frequency is not as rapid as predicted. Second, the model does not take into account the dynamics of copy number within a genome. Since most eukaryotic TE's occur 30 to 50 times per genome, crossing to an individual will still result in all of the offspring having a copy of the element due to chromosomal segregation (Chapter 1). Thus the element does not have to begin to transpose immediately in order to continue to spread through a population. This has the advantage that it allows the TE to have a more flexible control over copy number regulation.

In summary then, the models of Hickey (1982) and Ginzberg et al., (1984) both provide valuable insight into how transposable elements can spread in natural populations. Second, Ginzberg's model does provide a fairly accurate description of the population experiments described in Chapter 3. The next step would be to include the dynamics of copy number within the genome and self-regulation into a more comprehensive model.

In obligately sexual diploid organisms, TE's can spread even though they reduce the fitness of their host. In fact, the ability of P elements to reduce the fitness of their host was

what permitted their initial discovery. This is perhaps one of the reasons why it is more likely that one would see a TE like a P element in eukaryotes. Their negative effect will slow the rate of spread, as in the case of P elements, which slow their spread by inducing hybrid dysgenesis. In other words, given the ability of most TE's to self-regulate the only time one would notice the presence of a particular TE is between the time the element initially evolved and the time it took for the element to fix itself. Once fixed the element would be self-regulated and sequence divergence over time would result in the slow loss of it's ability to transpose. Therefore any eukaryotic TE that had a positive effect would spread extremely rapidly through natural populations, and once fixed would be recognized as simply another piece of noncoding DNA. This rapid spread followed by fixation of a sequence is the most likely explanation for the large amounts of noncoding DNA in eukaryotes (Doolittle and Sapienza, 1980; Orgel and Crick, 1980).

Evolution of Self-Regulation:

It has been shown be several authors that an equilibrium number of TE copies per genome can exist if transposition is self-regulated (Charlesworth and Charlesworth, 1983; Langley et al., 1983; Charlesworth and Langley, 1986). How important is the process of TE self-regulation and how will it affect the distribution and spread of TE's. Let us assume for the moment that that transposable elements have evolved as DNA parasites in

the cell (Doolittle and Sapienza, 1980; Orgel and Crick, 1980). In the extreme interpretation of this model, a transposable element may want to transpose at a high rate which will allow it to proliferate at its maximum potential. On the other hand, each transposition event runs a risk of introducing a detrimental mutation in the cell which would consequently be deleterious to the survival of the transposon. Therefore, one might predict that transposable elements would have evolved some sort of balance where the transposition rate could be maintained at a high level to ensure its survival and dispersal but not lead to a high risk of introducing multiple mutations or becoming a load on the replication machinery of its host (Johnson and Reznikoff, 1984).

There are several mechanisms of transposition self-regulation that have been well characterized. The repression system of Tn3 results from the regulation of transposase expression, which probably occurs at the level of transcription and translation (Heffron, 1983). Mutations in the tnpR gene (the repressor) lead to at least a ten-fold overproduction of transposase. This regulation appears to increase transposition frequency and to occur at the level of transcription because genes downstream from the transposase that are read through the transposase promoter show higher levels of transcription (Heffron et al., 1979). Expression of transposase is also limited by inefficient tranlation of the tnpA (transposase gene) transcript. Fusions between B-galactosidase

and the transposase show low amounts of B-galactosidase activity. Mutations selected for increased expression of B-galactosidase all improved a ribosome binding site in front of the methionine used for translation initiation of the transposase. Expression of the repressor gene tnpR is negatively regulated by its own product (Heffron, 1983). This is important in that there appears to be little zygotic induction of Tn3 on entering a virgin cell, presumably because the repressor is itself autogenously regulated and thus high-level transcription of the transposase is rapidly shut down (Heffron, 1983). A second type of transposition regulation is shown by Tn5 (Reznikoff, 1982). The functional unit of Tn5 is IS50R which encodes protein 1 (the transposase) and protein 2 (the repressor). Protein 2 alone is sufficient to inhibit transposase activity but not its synthesis (Johnson et al., 1982). Protein fusion experiments indicate that the transposition-inhibition activity cannot be explained by autogenous regulation of IS50R protein synthesis. Reznikoff (1982) favors a model whereby protein 2 inactivates protein 1 directly, perhaps by forming a mixed oligomer. Alternatively protein 2 could compete with protein 1 by binding to the transposase target sites or by sequestering some host component required for the transpsotion process. A third type of transposition self-regulation is demonstrated by Tn10 (Kleckner, 1983; Simons and Kleckner, 1983). Transposition of a single Tn10 element resident in the cell is reduced three to ten fold in the presence of a multicopy plasmid carrying IS10R (Foster et al.,

1981). Genetic analysis has shown: (1) that the region of IS10R that is both necessary and sufficient for inhibition corresponds to p-OUT and the template for its transcript. (2) Multicopy inhibition is due to a decrease in the expression of transposition functions; and (3) multicopy inhibition acts to depress translation but not transcription of the transposase gene (Kleckner, 1983). Simons and Kleckner (1983) proposed that inhibition of transposase translation occurs by direct pairing between the transposase mRNA and a small complementary, regulatory RNA specified by the IS 10-encoded pOUT promoter. In summary, these three prokaryotic transposons demonstrate self-regulation at (1) a transcriptional level (Tn3), (2) a translational level (Tn10) and (3) a post-tranlational level (possibly protein-protein binding) (Tn5).

Transposition repression is also known to occur in a number of eukaryotic systems. In a system analogous to transposition repression, replication of the yeast 2u plasmid has been shown to be regulated to maintain 50 to 100 copies of the plasmid in any cell. Under normal conditions, replication of the 2u plasmid is under strict control of cell cycle regulation. In other words, the plasmid population is doubled during the cell cycle by a single round of replication of each molecule in the cell (Zakian et al., 1979). Sigurdson et al., (1981) demonstrated that when a cell which formerly lacks 2u circles acquires a single copy of the plasmid, by the time the cell has grown into a colony, the average number cellular copy number of the plasmid has attained

the normal levels of 50 to 100 copies. Jayaran et al. (1983) proposed that two of the plasmid loci REP1 and REP2 constitute a copy control system that overrides normal cellular restrictions on plasmid replication and amplifies the plasmid when the copy number is low.

Robertson (1978) described a maize stock that generated 50-100 times the expected frequency of recessive mutations. This mutator activity was subsequently shown to result from a 1.4 kb transposable element Mu1. The Mu element of maize also shows transposition regulation. Robertson (1983) demonstrated that with increased doses of Mu, there is an initial increase in mutator activity, but the mutation frequency drops dramatically at higher levels of Mu. He assumed that this drop in mutator activity reflected a decrease in transposition rates. Allmand and Freeling (1986) demonstrated that when Mutator plants are crossed with an inbred line that does not have any copies of Mu, the progeny plants have approximately the same number of Mu sequences as did their Mutator parents. This maintenance of copy number can be accounted for by an extremely high rate of transposition of the Mu elements (10-15 transpositions per gamete per generation). Transposition of Mu and the events that lead to copy number maintenance occur very late in development of the germ cells but before fertilization.

Copy number regulation has also been shown for the P and I elements of Drosophila. In P lines the repressor prevents transposition whereas when a P male is crossed with an M female,

the lack of repressor in the M egg permits the production of transposase and allows transposition to occur. O'Hare and Rubin (1984) suggested that the P element repressor positively feeds back to stimulate its own activity. Once sufficient regulator had been produced, the regulator would shut off transposition. This model would explain the self-reproduction of cytotype and account for the fact that there is a sharp transition from low (M cytotype) to high (P cytotype) levels of regulator. However, the work of Laski et al. (1986) and Rio et al. (1986) provides us with a better understanding of P element regulation. The tissue specificity of transposition has been shown to result from splicing of message in the germ line cells. Therefore the change from M to P cytotype has to result from a decrease in spliced message relative to unspliced. This could result either from a change in the rate of splicing, or an increase in the amount of message.

What is interesting about these different systems of self-regulation, from a population geneticists viewpoint is that there is clearly strong selective pressure to evolve some form of self-regulation. Different transposons have solved the problem differently, but all of them have managed to evolve some form of self-regulation.

Models of Regulation:

The fact that transposable elements in eukaryotes exist in 30-100 copies per genome, and the numerous examples of

transposition self-regulation that have been demonstrated has led several authors to develop models for the evolution of self-regulation.

Doolittle et al., (1984) pointed out that if the average copy number of a transposable element in the cells of a host population increases, and each new transposition has the possibility of causing a lethal-mutation, eventually the host cells will begin to die faster than they are born, so the host cell population is driven to extinction and the element with it. On the other hand if the element acquires the trick of restraining its transposition, so that its copy number is limited, the host cells will continue to grow. Charlesworth and Langley (1986) examined the conditions under which self-regulated rates of transposition can evolve in populations of transposable elements infecting sexually reproducing hosts. The potential selective advantage to regulation was assumed to be derived from the deleterious effects of mutations associated with the insertion of newly replicating elements. They found that self-regulated transposition can easily evolve in hosts with low rates of genetic recombination per generation, such as bacteria. In this case there is a premium on regulating transposition because if a particular element does transpose and cause a deleterious muation, the TE will be completely linked to that element. However, conditions are more restrictive in organisms with free recombination. In this case, if transposition to a new site in the genome occurs, the new element may cause a deleterious

mutation upon insertion. However, the original copy of the TE can separate from the new deleterious copy by recombination. Thus any particular TE will not necessarily be linked to the mutations it causes when it transposes to a new site. When there is free recombination, the most promising model for the evolution of self-regulation involves dominant lethal or sterile mutations associated with transposition. A dominant lethal or sterile mutation in this situation would be similar to complete linkage, in that the element would be directly affected by any mutation of this nature that it causes.

How do these predictions match what has been found in the P-M system of hybrid dysgenesis. First, as one would expect, the development of cytotype occurs more slowly than the change in P element activity (Chapter 3). This makes sense from an evolutionary point of view. If an element is to be successful, it must first spread, and then change from an M to P cytotype to prevent over-replication and the destruction of its host. Second, as predicted by Charlesworth and Langley (1986), the nature of mutations in the P-M and I-R system involves sterile mutations. Therefore each active element is affected by its own transposition and recombination cannot separate the element from the negative effects it causes. Thirdly, regulation of transposition also occurs between different tissue types in the same organism. In organisms with a strict division between germ line and somatic tissue, transposition in somatic cells confers no competitive advantage to the TE, because there is no

possibility of transmission to the next generation (Charlesworth and Langley, 1986). The P and I elements fit this prediction exactly.

Finally, it should be noted that different eukaryotic transposable element have very different rates of transposition. For example, the Mu elements of maize appear to be able to completely replicate 10-15 copies per generation. In contrast, there are a number of elements that seem to transpose on an irregular basis and fairly slowly (eg: yeast Ty elements, Drosophila copia elements). P elements seem to be somewhere in the middle. They can increase in frequency fairly rapidly, as evidenced by Chapter 3, Kidwell et al. (1981) and Kiyasu and Kidwell (1984). However, they do not appear to be able to double their copy number each generation (Chapters 2 and 4). There seem to be reasons associated either with a particular element or the biology of the host organism that determine the rate at which an element can transpose and the nature of self-regulation. Future work will undoubtedly be directed towards understanding these differences.

Conclusion

This study was undertaken to examine how the transposable P element of Drosophila melanogaster can be maintained or increase in frequency in natural populations. Since the P element is known to cause sterility and mutations when it transposes, it has been suggested that the rapid spread of this element is entirely due to its ability to replicatively transpose. While previous

studies had been able to show an increase in the frequency of P-type flies in experimental populations (Kidwell et al., 1981; Kiyasu and Kidwell, 1984), in order to clearly demonstrate an increase in P elements in a population it was necessary to measure both P element activity and P element copy number.

In the population cage experiments described in this thesis, P element activity was found to increase very rapidly in frequency when starting with either 1% or 10% P-type flies. This increase in P element activity was matched by an equivalent increase in P element copy number. Therefore, P elements seem to be able to increase in these populations as a result of replicative transposition. The initial rapid increase in P element activity was not matched by an equivalent increase in cytotype. The cytotype of the population cages remained primarily M-type until at least generation 10, however once it began to change, the change occurred very rapidly.

In two separate sets of experiments, P-type flies were backcrossed to M-type flies using different mating schemes. It was found in all these experiments that the level of P element activity and the P element copy number decreased with each generation of backcrossing. However the different backcross matings had very different levels of P element activity, suggesting that the parental genotype of a fly has an important effect on the rate of P element transposition. It was demonstrated that this ancestral genotype effect was unlikely to have resulted from different amounts of P elements in the various

crosses, but it was difficult to provide a really adequate explanation for this effect.

Chapter 5 discussed several models of the evolution of transposable elements and compared these models with the results from this thesis. In particular the model of Ginzberg et al., (1984) provided quite a reasonable description of the experimental data, even though the model fails to incorporate several important parameters, namely copy number within a genome and self-regulation. In addition, the model of Charlesworth and Langley (1986) dealing with the evolution of self-regulation was discussed and it was pointed out how several of the predictions of their model closely fit the P-M system.

In summary, P elements are able to spread rapidly in laboratory populations presumably as a result of their ability to undergo replicative transposition. Thus they represent one of the best documented examples of selfish DNA, DNA that is able to survive because of its ability to maintain itself regardless of the effect it has on its host. Moreover, these elements offer an insight into the evolution and population genetics of all transposable elements.

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Appendix 2.1a: Percentage P element sequences compared to the Harwich stock using DNA hybridization, for experimental crosses at 27oC. (For calculation method, see Materials and Methods, Chapter 2).

Generation	Densitometer readings: XDH probe	Densitometer readings: P probe(HIII)	Ratio HIII/XDH	Percentage P elements
F3 A1	88.3	151.7	1.72	13.4%
F3 A2	96.4	189.2	1.96	15.3%
F3 B1	84.3	266.7	3.16	24.7%
F3 B2	132.7	284.7	2.14	16.6%
F6 A1	105.5	174.6	1.65	12.9%
F6 A2	70.0	91.5	1.29	10.1%
F6 B1	68.3	103.0	1.51	11.8%
Harwich	16.6	213.2	12.8	

Appendix 2.1b: Percentage P element sequences compared to the Harwich stock using DNA hybridization, for experimental crosses at 24oC.

Generation	Densitometer Readings XDH probe	P probe(HIII)	Ratio HIII/XDH	Percentage P elements
F3 A1	53.0	96.3	1.82	13.0%
F3 A2	120.0	238.3	1.99	14.3%
F3 B1	114.0	240.7	2.11	15.1%
F3 B2	138.7	296.8	2.14	15.4%
F6 A1	55.3	65.2	1.18	8.4%
F6 A2	54.0	104.7	1.93	13.8%
F6 B1	101.5	187.0	1.84	13.2%
F6 B2	156.5	232.3	1.48	10.6%
Harwich	6.3	319.4	13.96	

Appendix 2.1c: Percentage P element sequences compared to the Harwich stock using DNA hybridization, for experimental crosses at 21oC.

Generation	Densitometer Readings XDH probe	P probe(HIII)	Ratio HIII/XDH	Percentage P elements
F3 A1	202.3	208.3	1.03	12.2%
F3 A2	136.0	146.5	1.07	12.7%
F3 B1	80.9	91.0	1.14	13.5%
F3 B2	234.7	266.3	1.14	13.5%
F6 A1	147.3	25.1	0.17	2.0%
F6 A2	190.0	27.2	0.14	1.7%
F6 B1	123.5	19.3	0.38	4.5%
F6 B2	178.2	35.5	0.20	2.4%
Harwich	30.5	257.1	8.43	

Appendix 3.1a: Percentage of P-type flies (X + S.D., n=4) in the 1% Population cage experiments as measured by P element activity and P cytotype.

P element activity

Population A (Harwich)	Replicate			
Generation	1	2	3	4
5	24% + 35	24% + 32	16% + 24	21% + 26
10	50% + 29	35% + 38	37% + 35	52% + 42
20	86% + 31	32% + 41	82% + 40	60% + 55
Population B (π_2)				
Generation				
5	33% + 34	33% + 36	23% + 29	33% + 35
10	82% + 21	87% + 18	67% + 24	53% + 24
20	94% + 13	96% + 9	94% + 9	84% + 11

P cytotype

Population A (Harwich)	Replicate			
Generation	1	2	3	4
5	100% + 0	100% + 0	96% + 5	98% + 4
10	100% + 0	100% + 0	100% + 0	100% + 0
20	16% + 11	12% + 4	6% + 9	14% + 11
Population B (π_2)				
Generation				
5	81% + 37	95% + 11	95% + 10	96% + 7
10	82% + 21	87% + 18	66% + 24	52% + 24
20	0% + 0	4% + 9	12% + 16	8% + 13

Appendix 3.1b: Percentage of P-type flies (X + S.D., n=4) in the 10% population cage experiments as measured by P element activity and P cytotype.

P element activity

Population C (Harwich)	Replicate			
Generation	1	2	3	4
5	41% + 34	44% + 41	60% + 39	48% + 50
10	80% + 27	83% + 23	90% + 13	70% + 29
20	94% + 9	100% + 0	98% + 4	96% + 9
Population B (π_2)				
Generation				
5	26% + 33	50% + 40	49% + 38	27% + 40
10	46% + 21	57% + 36	36% + 30	29% + 25
20	64% + 17	88% + 16	46% + 34	60% + 36

P cytotype

Population A (Harwich)	Replicate			
Generation	1	2	3	4
5	86% + 25	91% + 25	98% + 4	98% + 4
10	45% + 50	51% + 39	34% + 41	44% + 45
20	3% + 6	7% + 6	4% + 9	12% + 13
Population B (π_2)				
Generation				
5	98% + 4	92% + 12	82% + 38	94% + 11
10	80% + 37	76% + 41	76% + 44	97% + 31
20	6% + 13	4% + 6	24% + 25	67% + 47

Appendix 3.2a

The measurement of P element copy number using DNA hybridization. These blots contain DNA from generation 2 of the population cage experiments. The upper blot has been probed with XDH and the lower blot with a P element probe (HIII). Each column (A through D) includes three DNA dots. In addition to containing DNA from the experiments described in this chapter, the samples in column C are from the population cage experiments described in chapter 4. Each population experiment has two replicates (eg: A1 and A2 are replicates from population A).

A1, A2 = Population A, replicates 1 and 2, 1ug DNA

A3, A4 = Population B, replicates 1 and 2, 1ug DNA

A5, A6 = Population C, replicates 1 and 2, 1ug DNA

A7, A8 = Population D, replicates 1 and 2, 1ug DNA

B1 to B8 are the same as A1 through A8 but are 250 ng samples.

C1, C3 = Population A, Chapter 4, reps. 1 and 2, 1ug DNA

C2, C4 = Population A, Chapter 4, reps. 1 and 2, 250 ng

C5, C7 = Population B, Chapter 4, reps. 1 and 2, 1ug DNA

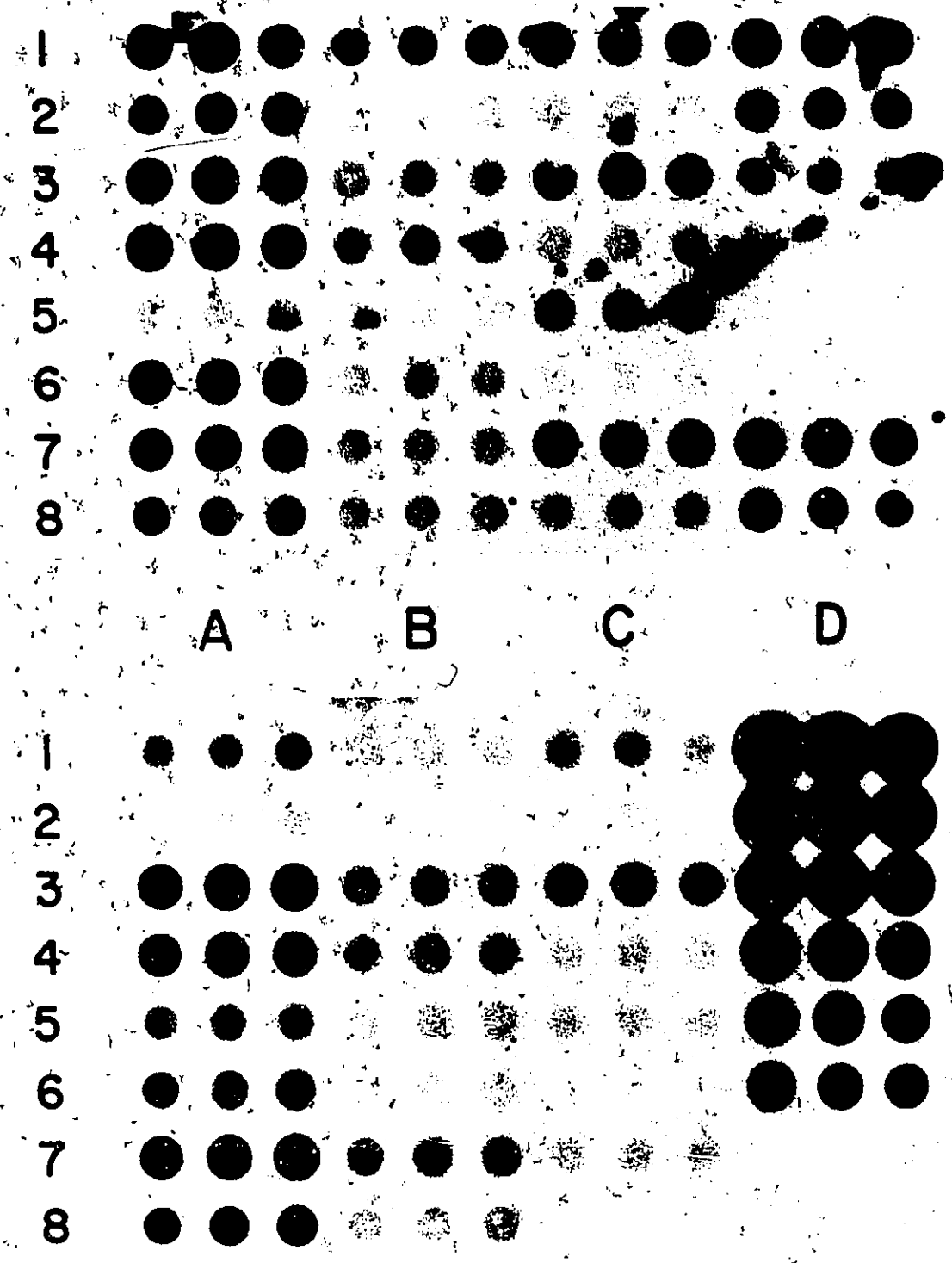
C6, C8 = Population B, Chapter 4, reps. 1 and 2, 250 ng

Samples D1 through D6 are a dilution series of Harwich DNA

D1 = 2 ug, D2 = 1 ug, D3 = 500 ng, D4 = 250 ng,

D5 = 100 ng, D6 = 50 ng.

Samples D7 = Canton-S, 1 ug and D8 = Canton-S, 500 ng.


1
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Appendix 3.2b

The measurement of P element copy number using DNA hybridization. These blots contain DNA from generation 5 of the population cage experiments. The upper blot has been probed with XDH and the lower blot with a P element probe (HIII). Each column (A through D) includes three DNA dots. In addition to containing DNA from the experiments described in this chapter, the samples in column C are from the population cage experiments described in chapter 4. Each population experiment has two replicates (eg: A1 and A2 are replicates from population A).

A1, A2 = Population A, replicates 1 and 2, 1ug DNA

A3, A4 = Population B, replicates 1 and 2, 1ug DNA

A5, A6 = Population C, replicates 1 and 2, 1ug DNA

A7, A8 = Population D, replicates 1 and 2, 1µg DNA

B1 to B8 are the same as A1 through A8 but are 250 ng samples.

C1, C3 = Population A, Chapter 4, reps. 1 and 2, 1ug DNA

C2, C4 = Population A, Chapter 4, reps. 1 and 2, 250 ng

C5, C7 = Population B, Chapter 4, reps. 1 and 2, 1ug DNA

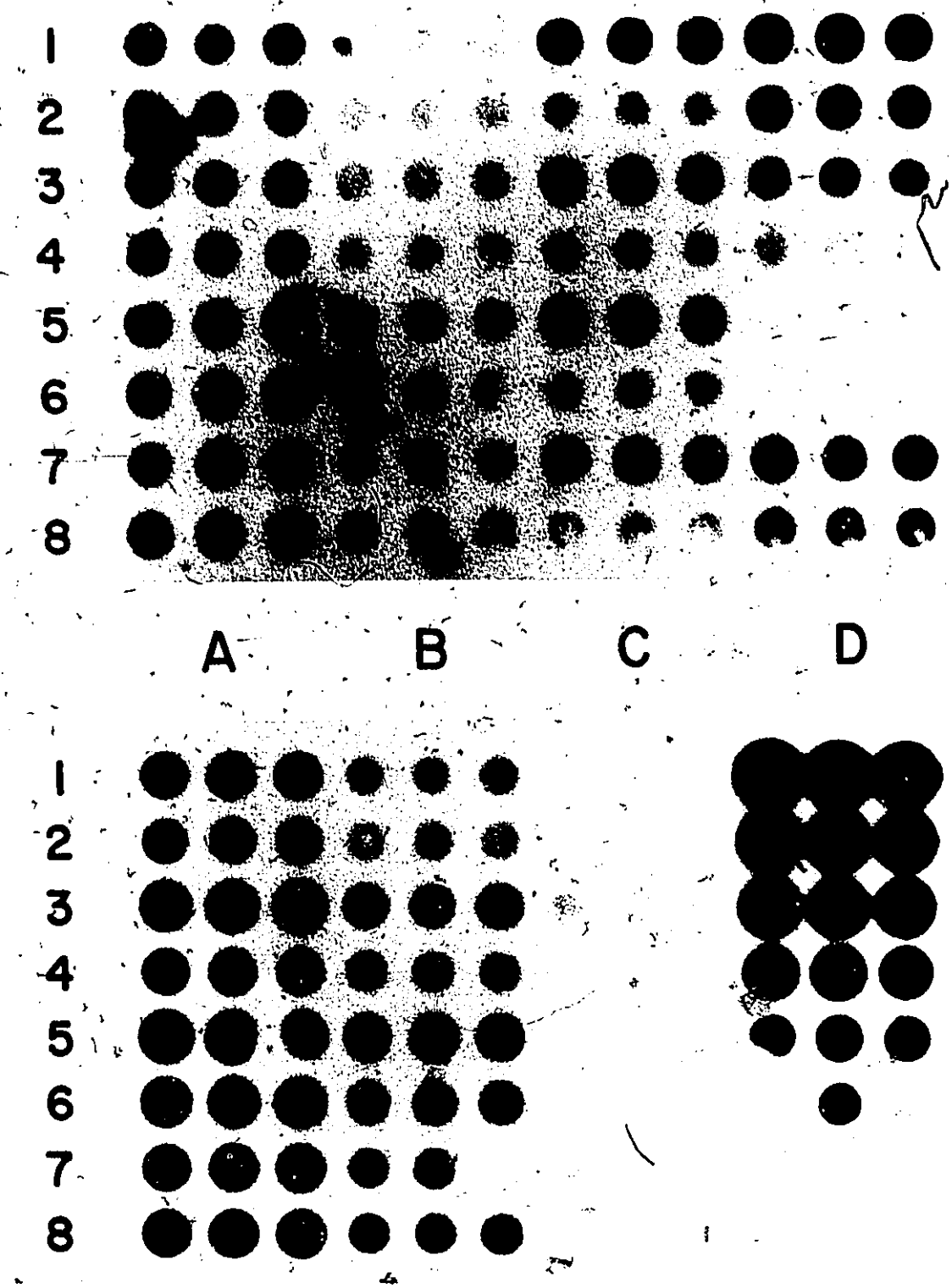
C6, C8 = Population Bb, Chapter 4, reps. 1 and 2, 250 ng

Samples D1 through D6 are a dilution series of Harwich DNA

D1 = 2 ug, D2 = 1 ug, D3 = 500 ng, D4 = 250 ng,

D5 = 100 ng, D6 = 50 ng.

Samples D7 = Canton-S, 1 ug and D8 = Canton-S, 500 ng.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C
D
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Appendix 3.2c

The measurement of P element copy number using DNA hybridization. These blots contain DNA from generation 20 of the population cage experiments. The upper blot has been probed with XDII and the lower blot with a P element probe (HIII). Each column (A through D) includes three DNA dots. In addition to containing DNA from the experiments described in this chapter, the samples in column C are from the population cage experiments described in chapter 4. Each population experiment has two replicates (eg: A1 and A2 are replicates from population A).

A1, A2 = Population A, replicates 1 and 2, 1ug DNA

A3, A4 = Population B, replicates 1 and 2, 1ug DNA

A5, A6 = Population C, replicates 1 and 2, 1ug DNA

A7, A8 = Population D, replicates 1 and 2, 1ug DNA

B1 to B8 are the same as A1 through A8 but are 250 ng samples.

C1, C3 = Population A, Chapter 4, reps. 1 and 2, 1ug DNA

C2, C4 = Population A, Chapter 4, reps. 1 and 2, 250 ng

C5, C7 = Population B, Chapter 4, reps. 1 and 2, 1ug DNA

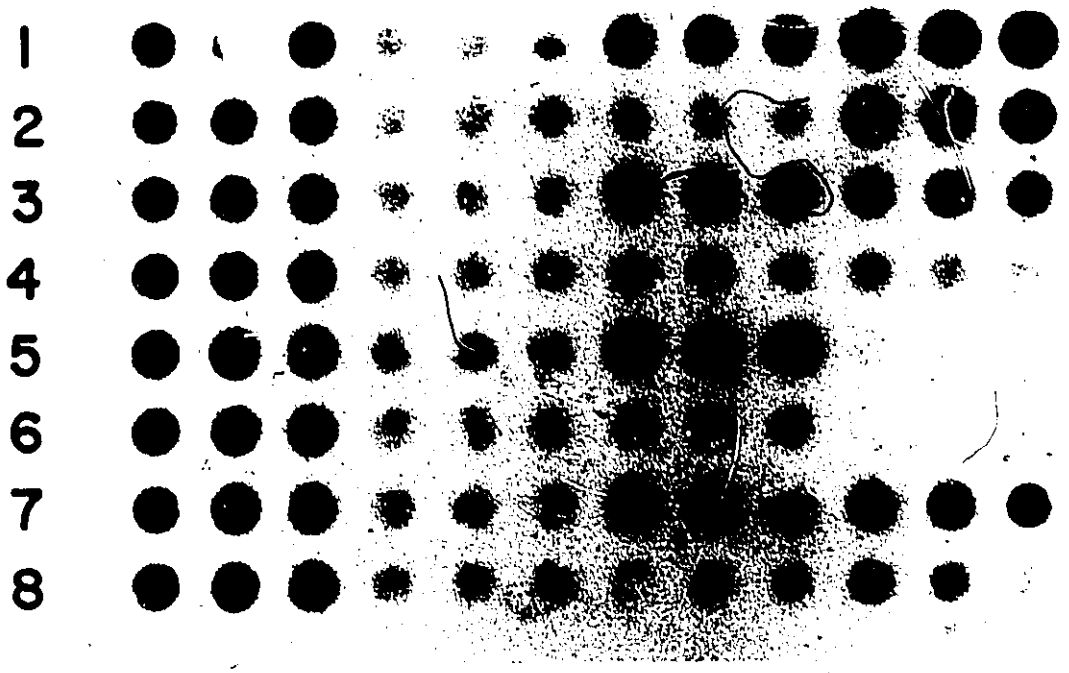
C6, C8 = Population B, Chapter 4, reps. 1 and 2, 250 ng

Samples D1 through D6 are a dilution series of Harwich DNA

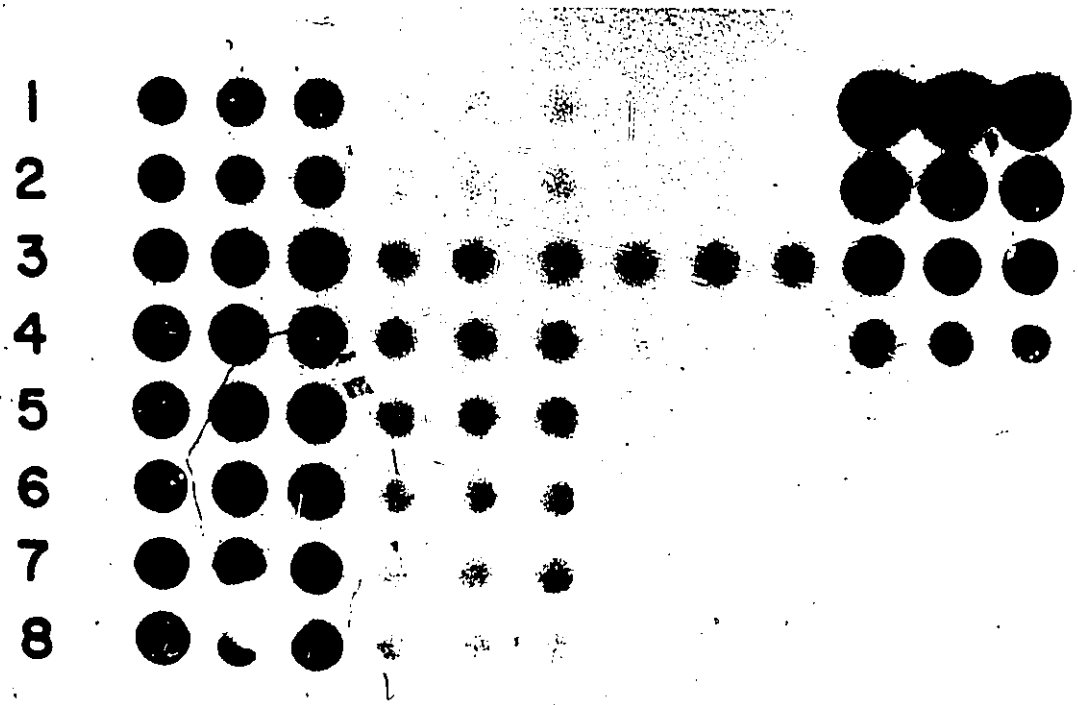
D1 = 2 ug, D2 = 1 ug, D3 = 500 ng, D4 = 250 ng,

D5 = 100 ng, D6 = 50 ng.

Samples D7 = Canton-S, 1 ug and D8 = Canton-S, 500 ng.



A B C D



Appendix 3.3a: Percentage P element sequences compared to Harwich using DNA hybridization, for 1% population cages.

Generation			Densitometer Readings XDH probe	Densitometer Readings P probe	Ratio HIII/XDH	Percentage P elements
2	Harwich	1	52	45	0.87	3.5%
		2	26	17	0.64	2.5%
	π_2	1	79	184	2.33	9.2%
		2	87	156	1.79	7.1%
5	Harwich	1	29	213	4.91	27.9%
		2	21	195	5.30	30.1%
	π_2	1	62	216	3.58	20.3%
		2	32	234	5.14	29.2%
10	Harwich	1	189	113	0.60	25.1%
		2	55	83	0.74	31.1%
	π_2	1	184	234	1.27	53.4%
		2	122	174	1.41	59.1%
20	Harwich	1	143	159	1.11	59.4%
		2	135	153	1.13	60.7%
	π_2	1	144	242	1.67	89.8%
		2	159	254	1.60	86.0%

Appendix 3.3b: Percentage P element sequences compared to Harwich using DNA hybridization, for 10% population cages.

Generation			Densitometer Readings XDH probe	P probe	Ratio H111/XDH	Percentage P elements
2	Harwich	1	18	45	2.48	9.8%
		2	81	65	0.78	3.1%
	π_2	1	85	191	2.25	8.9%
		2	54	105	1.95	7.7%
5	Harwich	1	74	163	3.19	18.1%
		2	99	222	2.18	12.4%
	π_2	1	46	110	4.26	24.2%
		2	89	141	2.38	13.5%
10	Harwich	1	96	163	1.69	71.5%
		2	50	83	1.64	68.9%
	π_2	1	249	249	1.00	42.0%
		2	303	276	0.97	41.0%
20	Harwich	1	182	247	1.36	73.1%
		2	170	231	1.36	73.1%
	π_2	1	167	216	1.57	84.4%
		2	166	219	1.32	71.0%

Appendix 4.1: Percentage of P-type flies (X + S.D., n=4) in the 10% Dilution Population cage as measured by P element activity and P cytotype.

P element activity

Population A (Harwich)	Replicate			
Generation	1	2	3	4
5	0% + 0	0% + 0	0% + 0	4% + 6
10	0% + 0	2% + 6	11% + 19	17% + 26
20	0% + 0	0% + 0	6% + 9	6% + 9
Population B (π_1)				
Generation				
5	0% + 0	0% + 0	0% + 0	8% + 11
10	0% + 0	0% + 0	1% + 3	1% + 3
20	2% + 5	0% + 0	2% + 4	0% + 0

P cytotype

Population A (Harwich)	Replicate			
Generation	1	2	3	4
5	100% + 0	100% + 0	100% + 0	100% + 0
10	100% + 0	100% + 0	100% + 0	100% + 0
20	96% + 5	100% + 0	100% + 0	100% + 0
Population B (π_2)				
Generation				
5	100% + 0	100% + 0	100% + 0	100% + 0
10	100% + 0	100% + 0	100% + 0	100% + 0
20	100% + 0	100% + 0	100% + 0	100% + 0

Appendix 4.2: Percentage P element sequences compared to Harwich using DNA hybridization, for 10% Dilution population cages. Samples 1 and 2 are from replicate cages of the same experiment.

Generation		Densitometer Readings XDH probe	P probe	Ratio HIII/XDH	Percentage P elements
2	Harwich 1	115	20	0.17	0.7%
	2	87	89	1.02	4.0%
	π_2 1	18	0	0	0%
	2	103	4	0.17	0.2%
5	Harwich 1	112	0	0	0%
	2	88	1	0	ç0.1%
	π_1 1	70	0	0	0%
	2	66	0	0	0%
10	Harwich 1	79	0	0	0%
	2	91	2	0.02	ç0.5%
	π_2 1	114	0	0	0%
	2	94	0	0	0%
20	Harwich 1	140	0	0	0%
	2	147	36	0.24	13.1%
	π_2 1	177	0	0	0%
	2	131	0	0	0%