

HYBRID DYSGENESIS AND P ELEMENTS

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1. HYBRID DYSGENESIS

1.1. Abnormal mutation.

In the chapter entitled 'Fitness in *Drosophila*', I have written about

Kidwell from Brown University, and she gave a talk about mutation effects in her experiments which was eerily similar. Jim Crow wrote an article in the 'Perspectives in Genetics' series entitled 'The Genesis of Dysgenesis' [3], mentioning this meeting, which according to his account was in 1972, although I find it hard to believe it was as early as this. Anyway as a result of this meeting I began a very rewarding collaboration with Margaret, and also with her husband Jim Kidwell, that lasted for several years.

1.2. Male recombination.

Somewhat earlier than the above meeting, Hiraizumi had reported [11] that chromosomes from a wild population can induce recombination in males. This may not sound particularly surprising, but it was a well established fact in *Drosophila* and other insects that males lack the machinery for recombination. So something abnormal was clearly going on in these flies. The rate of recombination was only of the order of 1% or less for loci at the opposite ends of a chromosome arm, compared to nearly 50% recombination in females, but nevertheless of was quite significant compared to what was found in laboratory strain flies.

Some time after the meeting in Amherst, Margaret Kidwell reported to me that the flies from her lines also induced some male recombination. I'm not sure prior to that Hiraizumi hadn't also reported mutation effects in his lines. I tested the Hunter Valley flies and found that they, too, caused recombination. Much of the latter part of this chapter is devoted to experiments on how this recombination occurs.

It is much easier to measure male recombination than to measure mutation. So I started to do experiments on recombination rather than mutation. The basic design of the experiment that we did is shown above. It involves simply collecting wild type flies and crossing them to a stock that contains three recessive chromosome 2 markers, located respectively at the left end of the chromosome, just beyond the centromere, and close to the right end.

The most useful of these markers, *cn* and *bw*, are eye colour markers. The wild type eye colour is red, while the combination of *cn* and *bw*, both in homozygous condition, leads to a white eye. Recombinants are either + *bw*, which is a brown eye, and *cn* + is 'cinnabar', a bright orange-red colour. Such eye colour variants are very easily spotted and scored. The *al* 'aristaless' marker is only slightly more difficult.

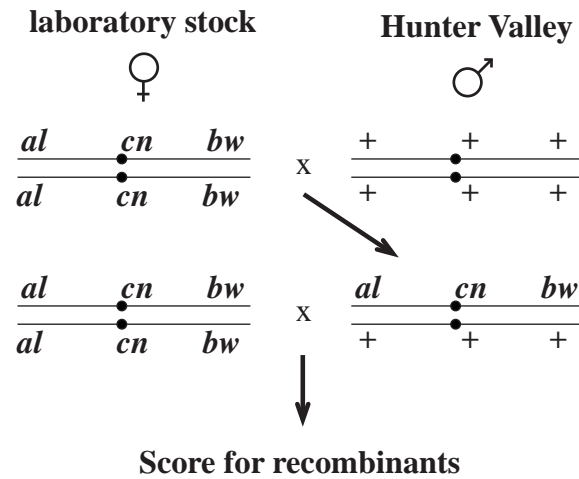
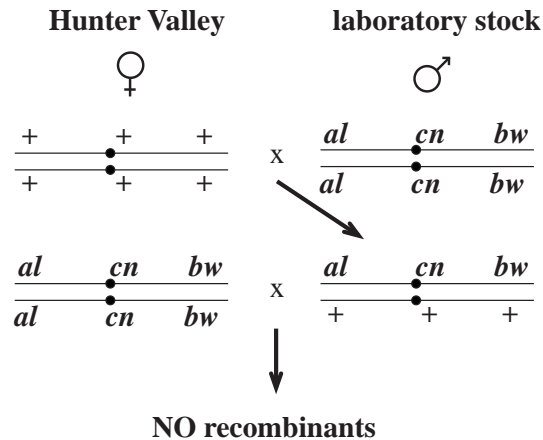


FIGURE 1. Two-generation cross for detecting male recombination

We did many experiments of this kind to try to describe the pattern of recombinants. I'll describe some of these experiments later [29].

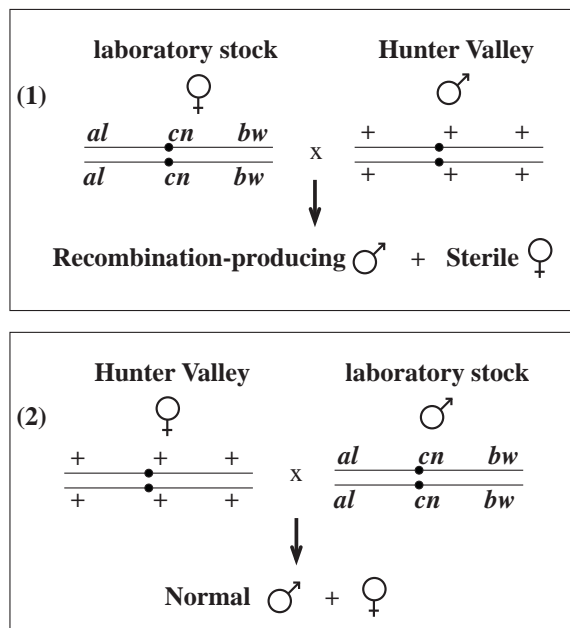
1.3. Margaret Kidwell's breakthrough.

I must have been out of contact with Margaret for a year or so when a letter came from her describing two important things she had found. The first was that if one did the initial cross the opposite way around, nothing happened.



The second thing she found was that there was sterility, primarily in females, from the first cross, the one in which wild type flies were used as the male. No sterility was found when the wild type flies were used as the female. The properties of sterility and recombination

went together, as shown below. These findings were published shortly afterwards [13].



These findings were obviously critical. We had assumed to this point that there was something in the wild type flies that produced recombination, just that we couldn't measure it until we crossed to a strain with the necessary recessive markers. The non-reciprocal nature of the phenomenon showed that what was going on in the male flies that produced recombination was not just a product of the wild type strain(s). Rather it was a phenomenon of hybrids between such strains and the laboratory strain.

Furthermore the presence of non-reciprocal sterility connected the phenomenon that we were studying to something that had long been known in both plants and animals. Curiously the sterility in Margaret's case went the opposite way from what is known as 'Haldane's rule' where in cases of sterility in one sex but not the other, it is usually the heterogametic, XY, rather than the homogametic, XX, sex that shows sterility.

I was immediately able to confirm the non-reciprocal nature of male recombination using our Hunter Valley stocks. There was rather less sterility, however, than in the crosses using her strain called Harwich. Shortly afterwards a colleague, Don Angus, found a strain in an area close to Adelaide called ParaWirra. I started using the strain, which

was at least as powerful as Harwich in producing sterility, including some male sterility. In my youth the area where this strain was collected was called Humbug Scrub. We ought to have used this name rather than the sanitised ParaWirra.

In retrospect it shows a lack of imagination that I had not found the non-reciprocal effect myself. We must have done the crosses of Figure 1 hundreds of times under different conditions of temperature, aging etc, but it had never occurred to me to do the initial cross the other way around. The way we did the cross was the easy option. It is simpler to keep a large laboratory strain going, such the the *alcnbw* strain, and to select 'virgin' females from this, than it is to select virgin females from the wild or from their immediate offspring.

1.4. Hybrid dysgenesis.

It was by now pretty obvious that something was going on that affected germline development in the hybrids of crosses between laboratory and wild strains. Margaret and I searched for terms to describe such a phenomenon and came up with 'Hybrid dysgenesis', a term that caught on quickly in the field. We thought we had invented the term but were surprised some time later to see that it was already listed in the Oxford dictionary, although the entry was rather vague about its manifestations.

We also introduced the terms P (for Paternal) and M (for Maternal) indicating the direction of the cross that produced dysgenesis. In Figure 1, therefore, the Hunter Valley strain is the P strain and the *alcnbw* strain is the M strain. As mentioned below, it turned out subsequently that Margaret and I had somewhat different ideas about what P and M meant.

We worked quite diligently to identify all the things that went wrong in dysgenic hybrids, which turned out to be almost any aspect of genetics that we could think of including male and female sterility, elevated mutation rates, non-disjunction, recombination rates etc. This was written up for publication, and the paper eventually appeared in 1977 [14].

1.5. Georges Picard and the I-R system.

In 1976 a paper by Georges Picard from a group working in Clermond-Ferrand appeared in Genetics describing a phenomenon, also in crosses between different laboratory strains and wild type strains, in which females were sterile, but for a rather different reason than Margaret

and I typically found. Our hybrid females laid a much reduced number of eggs, whereas Picard's females laid the normal number of eggs most of which failed to develop. The non-reciprocal nature of the cross was the same. The sterility came only when the I strain was used as the male parent and the R strain as the female parent, rather than vice versa.

We hadn't been aware of the relevance of the French group until the Genetics paper appeared, but there were earlier publications in French, which naturally we hadn't seen, plus some early turgid accounts in the 'trade journal' *Drosophila Information Service*. So by the time the Genetics paper came out they were years ahead in their analysis. Most importantly they had mapped the source of the 'I' properties and had found that it jumped around from chromosome to chromosome, a phenomenon they called 'Chromosomal contamination'. They also found strange heritable effects of aging of the R strains.

It didn't take too long for us to show that chromosomal contamination was also a feature when we tried to map the chromosomal location of the causes of the P properties. Now we know that this jumping around from one chromosome to another is a feature of transposable elements, and that a particular element, the P element, was responsible in our case. In retrospect we ought to have been thinking about such elements, given that they had been characterised by McClintock in maize many years earlier. I was particularly blinded in this case by being focused on another hypothesis to explain hybrid dysgenesis in terms of 'spatial organisation of chromosomes' [24]. I'll go into this story a little later in this account.

The relationship between our work and the work of the French group worried me a lot. We had noted many aspects of the breakdown of genetics in the hybrid, while they had only one. Nevertheless the similarities were such that it was clear that they were closely related phenomena. Margaret's intuition in this turned out to be much more accurate than mine. She simply decided that two independent systems were involved, the P-M and I-R systems. I found it difficult to see the two as independent, particularly since I had found quite a mixture of the two types of female sterility in my crosses involving the Hunter Valley strains. I had thought that we were introducing the terms P and M as generic descriptors, but now they were being applied to in a much more specific way.

One other puzzling aspect of the hybrid story should be mentioned here. All M strains, those that acted as female parent when dysgenic

was produced, were laboratory strains, descendants of flies collected in the 1930s and earlier. The P strains, by contrast, were all recent collections. Either the M strains or the P strains had to have changed. From first principles it seemed to me much more likely that the changes were in laboratory strains. After all these were the ones that had been under unnatural conditions. Why should ordinary wild strains have changed over 50 years or so, scarcely a period long enough for evolutionary changes to have occurred. Had atom bombs or something changed the environment?

Obviously in retrospect my intuition was wrong (yet again). Why changes had occurred in wild strains now seems explicable in terms of recent worldwide spread of *D. melanogaster* bringing it into contact with P element-containing species [4]. But it still seems remarkable to me that not only the P element, but possibly the I and other elements have invaded in such a short time.

2. P ELEMENTS

2.1. Gerry Rubin and isolation of the P element.

Margaret's strains had been used to produce mutations at the *white* locus [19]. Then in remarkably quick succession, Gerry Rubin and colleagues were able to clone the *white* locus, to use this DNA to probe the mutant strains and recover fragments of a transposable element insertion causing the mutation, and then to use these fragments to recover the complete 'P element' [2].

These experiments were then followed by others to characterise the element, and to show how it can be used to transform flies with inserts of any desired DNA [21]. It is this latter use that has led P elements to become the workhorse of *Drosophila* genetics, with many thousands of strains now available for developmental and other genetical studies.

2.2. David Finnegan clones the I factor.

The I properties in the I-R system also turned out to be a transposon, as predicted much earlier by Picard et al. The group in Clermond-Ferrand collaborated with David Finnegan's lab in Edinburgh to clone the I element (factor), which turned out to be a much harder job than the initial cloning of the P element. The I element is a LINE element, somewhat longer than the P element, and more difficult to manipulate in crosses.

Although I had no role in the cloning of the I element, I had a sabbatical leave in David Finnegan's lab in 1984 when the work was still going on. I went there to learn to be a molecular biologist, under the guidance of David and his partner Helen Sang. I had a great time cloning, doing Southern blots etc, and felt that I was a fully-fledged molecular biologist at the end of six months. Unfortunately little of this transferred to Sydney University when I went back and attempted to set up a molecular lab to work on P elements.

2.3. Bill Engels comes onto the scene.

Shortly after the Kidwell et al paper appeared in 1977 [14], a paper was published by Bill Engels [7], then a graduate student at the University of Wisconsin. This was the first in a series of papers that over the next 20 years transformed the study of P elements. Bill Engels and his associates, originally just Christine Preston, showed clearly that specific breaks were introduced, frequently at the site of P elements. The rules of maternal inheritance were clarified. A remarkable new sensitive mutation for identifying dysgenic mutations, *sn^w* was identified. All these, and other results from Bill's, plus other labs, were summarised in a comprehensive review [8].

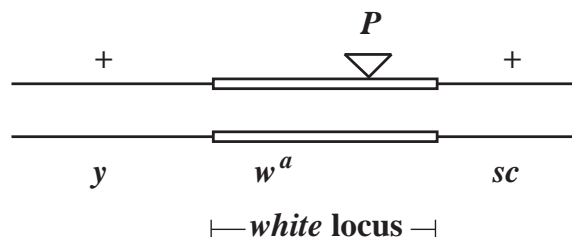
Even more important contributions came later, once transformation of P elements had been established. Bill's lab identified a stable source of P element 'transposase' labelled $\Delta 2-3$ [18]. This could be manipulated in crosses, so that P elements could be alternatively made active or inactive. The extensive use of P elements in *Drosophila* genetics over the past 20 years would have been impossible without the strains that Bill and his collaborators have provided.

2.4. How P elements multiply.

The P element is a reasonably short element, 2907 bp long. It appears to code only a single gene, a 'transposase' that allows the element to excise itself [1]. How can a single gene such as this be responsible for all the functions needed for the element to increase its numbers in the population? Bill solved this problem in a study in which I had the good fortune to be involved [5].

I spent a sabbatical leave in Bill's lab in 1988. This was an exciting time when single P element mutations, and the means of manipulating them, had just become available. I tried a number of experiments, including one which I'll go into in a bit of detail.

I was interested to see whether P elements might transpose themselves to exactly homologous positions on the chromosome. There were a couple of stocks involving the *white* locus that enabled this experiment to be done. One of these was a P element insertion into the locus causing white eyes. The other was a different mutation, white-apricot, a light orange colour. This was available in a strain with 'flanking markers', two mutations quite close to the *white* locus.



Females were produced which had one copy of each mutation, as in the diagram. They themselves had light orange eyes. What I was looking for was male offspring where the P element had jumped over into the opposite chromosome, thereby giving offspring that were white-eyed and also had the *y* and *sc* flanking markers.

The experiment was a failure, as most exploratory experiments seem to be. No flies of this type showed up. Most were either plain white, or alternatively orange with the *y* and *sc* markers, as would be expected if nothing untoward happened.

Although I am fairly unobservant, I couldn't help notice, however, that amongst the progeny were a few red-eyed flies. I wasn't all that surprised about such flies. If the P element jumps out, leaving the *white* gene intact, that seemed to be exactly what one would have expected. But I happened to be discussing the experiment with Bill Eggleston, a graduate student in the lab. He pricked up his ears, because it turned out that he had been trying to jump the P element out of exactly the same *white* mutation with no success at all. We compared notes, and it became clear that the difference between our experiments was that he was trying to jump the element out in males while I was doing it in females.

There was an obvious explanation for this difference. I had not thought about it before, but if the P element jumps out and leaves a gap that is not properly repaired, then any progeny containing such a mutation will be indistinguishable from the original mutation. If, however, the gap can be repaired using normal DNA, then it is possible to restore

the original gene and produce red eye colour. The apricot mutation is, fortuitously, in a different part of the gene. So all the ingredients for making up a normal gene were there in my experiment. In Bill Eggleston's male flies, however, there was only a single X chromosome with no normal DNA opposite the insertion to repair from.

We reported this result at a lab meeting. Both of us were involved in other experiments, and went our separate ways. It was Bill Engels who saw the critical interpretation of this result. He asked what is expected to happen if a P element jumps out *after* DNA replication has occurred (see Figure 2).

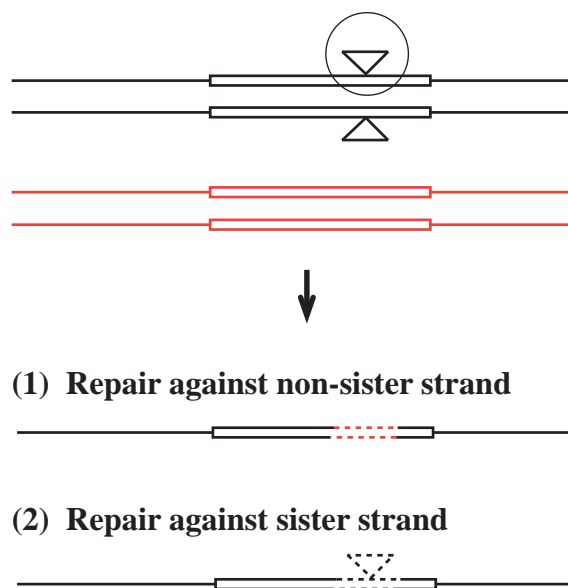


FIGURE 2. Two types of repair possible after P element (circled) jumps out

The first outcome restores the gene to its normal state. These are the ones that we see. The second, probably the more frequent occurrence but the ones we don't see, involve repair against the sister strand containing the same insertion. This restores the copy number of the element. Provided that the excised element inserts elsewhere in the genome, the copy number has increased.

The model of replication is often called the cut-paste model, although it would be more accurate to call it the cut-copy-paste model. It has proven difficult to get evidence that P element jumps are timed to occur after DNA replication [32]. But it is such a nice model that I find it

hard to believe that it is wrong, for P elements and perhaps for other transposable elements that don't have an RNA intermediate.

The experiment as written up [5] contained none of our initial observations. Bill and Dena Johnson-Schlitz in the lab repeated the same sorts of crosses under much more carefully controlled conditions. It was the start of Bill's move out of P element biology and into the more important field of DNA repair, where his work has continued to be at the forefront for many years.

I'd like to mention one more experiment from Bill and his group that shows that the repair doesn't need to be from a sequence at the exact homologous site [6]. They made double-stranded breaks in an X chromosome which could be repaired by a homologous sequence that they inserted at various places in the genome. They found that the repair could occur at some frequency no matter where the homologous insertion was located, although there was a preference for X chromosome sites, especially for ones linked to the breaks even at long distance. It tells a lot about the likely spatial organisation in the nucleus, and how there must be a homology searching going on that affects the entire genome.

3. THE MECHANISM OF MALE RECOMBINATION

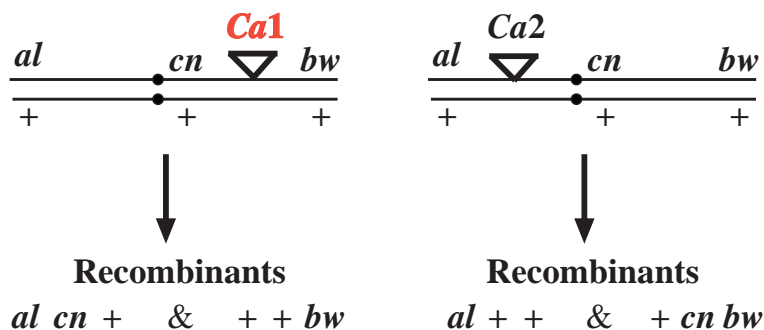
My interest in the field, following the sabbatical leave in the Engels lab in 1988, has been almost entirely in trying to understand how P elements cause recombination. It has been a long haul, but I believe that we now understand most of this.

3.1. Male recombination caused by GM elements.

The starting point of this work was a series of insertions of a genetically modified element known as CaSpeR. This was constructed in a lab in Italy to be a vector for introducing genes into the genome by transformation. It contains a 'mini-white' gene, that transforms the colour of *w* mutants into a light-orange colour. Just at the time I arrived there, a whole series of these CaSpeR elements had been transformed into the genome and their positions mapped by Randy Phillis in the Engels lab.

I concentrated on two elements, the first inserted into the right arm of chromosome 2 and the second into the left arm. Using the markers *al*, *cn* and *bw* shown in Figure 1, it was clear that the first one led to most

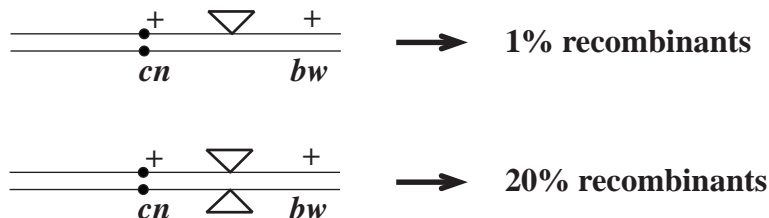
recombination in the right arm of the chromosome and the second one correspondingly in the left arm [26] (see diagram below).



The rates of recombination, 0.5 - 1%, were low, but not appreciably lower than given by whole M x P crosses such as shown in Figure 1. This was therefore a considerable advance over using P strains, with their mixture of unknown numbers of P elements. The CaSpeR element is not capable of jumping or causing recombination by itself, but needs a second 'transposase source' P element for its activation, the Δ 2-3 element.

3.2. The surprising effect of homologous elements.

The remainder of the work concentrated on the insertion in right arm designated as *Ca1*, inserted in the 50C region. We produced a stock that was homozygous for the element, including outside markers *cn* and *bw* to measure recombination, and the Δ 2-3 element to make the *Ca1* element active. Huge increases in recombination were seen [25], greater than had ever been found in male recombination. It was an exciting result, even though there was no simple explanation. It did, however, strengthen the suggestion that the actual site of the element was likely to be involved in recombination events, rather than triggering some event distant from the element.



3.3. Cloning of the *Ca1* region.

Now the hard work began. In order to see what was going on we really needed the DNA sequence surrounding the element. Nowadays with the complete *Drosophila* sequence available, and the availability of cheap PCR primers, this would be a simple task. In those days it involved phage λ cloning, sub-cloning and hand sequencing. Most of the original work was done by Leila Blackman, following a short stint by Carolyn Byrne who initially got the lab started around 1990. Leila left before the sequencing was completed and it was taken over by Yasmine Svoboda.

Enough sequence was available by about 1995 to allow PCR primers to be designed. These are shown in the diagram below. Two of the primers, labeled A and D were in DNA flanking the *Ca1* insertions. The element itself, and primers B and C close to the ends, are shown in red.

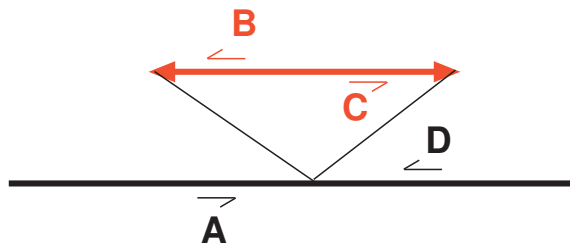


FIGURE 3. PCR primers defining the *Ca1* element insertion.

The complete sequence took some time longer. We were able to show that the *Ca1* insertion lies in an intron of the cystine proteinase gene, and to relate the sterility and lethality of various deletions to this and neighbouring genes [9].

3.4. Modification of the *Ca1* element.

The high rate of recombination given by homologous elements was intriguing. I wanted to see if the size of the elements was critical in this process - I don't even remember quite why. I think it was Bill Engels who suggested during a visit to Australia that I try putting the elements through a generation of dysgenesis to try to produce some internal deletions in the element. This turned out to be a critical experiment, although as usual for reasons other than the expected ones.

We made up male flies containing the *Ca1* element and the $\Delta 2-3$ element. We selected 64 non- $\Delta 2-3$ offspring that no longer had the orange

eye CaSpeR phenotype, indicating that they had suffered some sort of deletion. This could have been anything from a few bases up to the complete element.

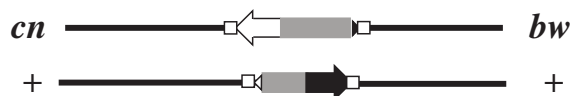
Exact deletions, those expected if the element has been deleted and the region repaired, were detected by the presence of an A-D band of characteristic size. Similarly elements that had both the A-B and C-D bands must have had internal deletions. Most progeny were of one of these two types.

In addition we found a few elements that had either only the A-B band or only the C-D band. The elements with just the A-B band must have effectively just a left end, so that some or all of the right end had been deleted. Similarly the elements with just C-D bands must have just a right end. Sequencing showed that in most cases the deletion had left behind around 15 bases of the actual element end. The Beall and Rio model [1] in which P element transposase acts as a giant restriction enzyme producing 15bp overhangs, suggests why this form of deletion might be quite frequent, luckily for us as it turned out.

When these end-deleted elements were made homozygous, none of them gave any recombination at all. Clearly none of them were functional, a result predicted from earlier experiments in the Rubin lab. Although P elements contain a 31bp inverted repeat at the ends, these experiments showed that left ends and right ends are different, due to sequence differences beyond the 31bp, and that functional elements need a complete left end and a complete right end.

3.5. Formation of hybrid elements.

Although initially we paid little attention to these elements with only one functional end, there was one experiment that seemed worth doing with them. What would happen if we put a left-end element and a right-end element into the same fly? It seemed a long shot, but nevertheless we set up males having the male genotype shown in the figure below. In addition to the second chromosome genotypes shown, the flies also contained the $\Delta 2-3$ element.

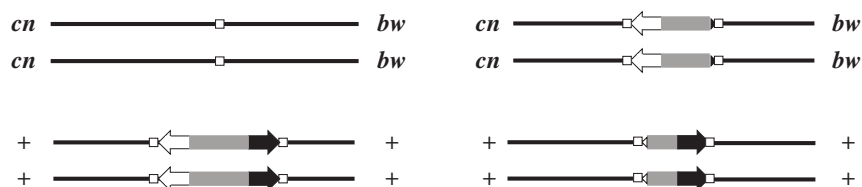


To our considerable surprise and delight, the recombinants poured out, at a rate greater than any we had seen in any experiment in the past

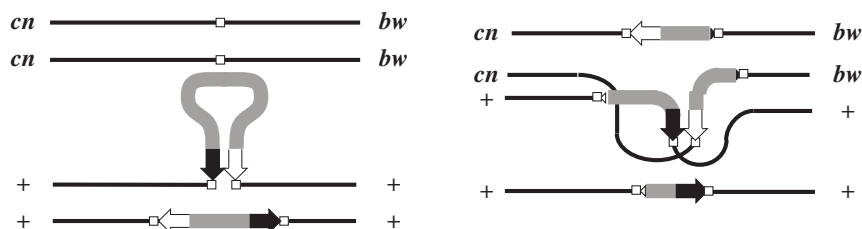
[30]. The only possible interpretation seemed to be that despite being on different chromosomes, the left and right ends must be getting together to form some sort of functional 'hybrid element'. Note that 'hybrid' here has nothing to do with the 'hybrid' in hybrid dysgenesis.

3.6. Consequences of 'excision' of a hybrid element.

The formation of a hybrid element can be pictured as below. The chromosome set on the left shows the situation after a normal element has duplicated. On the right is the equivalent situation after duplication of the genotype pictured previously with two end-deleted elements.

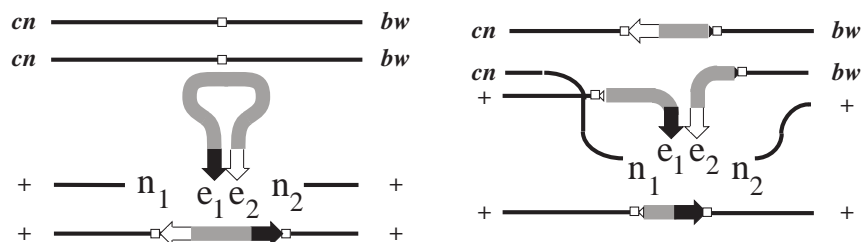


The second stage pictures the association of the ends of one of the normal element (left), and the association of two end-deleted elements (right).



The third stage shows excision of the element. On the left, the element ends e_1 and e_2 are shown as free ends prior to insertion elsewhere in the genome. In fact it is likely that the ends never exist as free ends, but are instead inserted to the new genome site within a 'transpososome' structure. The ends that do not contain elements, n_1 and n_2 , are presumably now subject to repair in either of the ways depicted in Figure 2, either restoring the element or completely excising it.

The equivalent 'excision' on the right is somewhat more complex. The process at the element ends is pictured as being equivalent to that of a normal element. However in this case there cannot be true excision since the two ends, rather than being bound to each other, are bound to different chromosomes. Joining of e_1 and e_2 via insertion into a new site, or joining of n_1 and n_2 via repair, are both expected to lead to recombination.



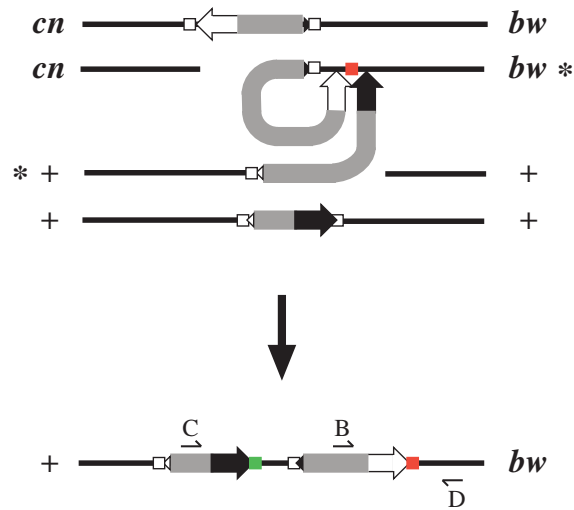
3.7. Insertion leads to $+ bw$ recombinants and repair to $cn +$.

A surprising, but very useful feature of the crosses, is the fact that there are two very different processes, both leading to recombination, but to different genotypes. The joining of n_1 and n_2 is easier to see. Repair against either the right or left element is expected. The results are in agreement with this, with a majority of repair against the shorter of the two elements, as might be expected.

The consequences of insertion are much more complicated. Mark Tanaka, when he was an Honours student, worked out all the possible insertion classes, depending on which strand the insertion occurs and the position with respect to the original insertion site (Gray et al, 1996, Figure 2) [10]. Some lead to inviable products, but the majority lead to $+ bw$ recombinants with various insertions and deletions.

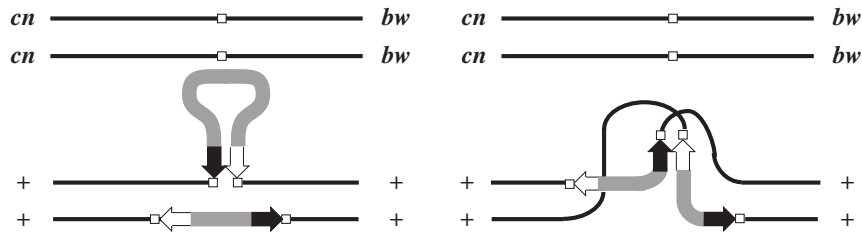
Our favourite recombinant is shown in the next figure. It involves insertion back into one of the chromosomes involved in the initial association. Both elements are present in the final gamete. Such recombinants can be readily detected by PCR. The B-D primer pair normally point in the same direction rather than towards each other, as shown in Figure 3. Now they produce a fragment which could only be due to a rearrangement of elements such as shown.

The really significant aspect is the unequivocal sign that the recombinant is an insertion rather than just a breakage and rejoin. When P elements insert, they duplicate an 8bp sequence that appears at both ends of the insertion. The original 8bp insertion associated with *Ca1* is shown as a small white box in most of the diagrams. When the hybrid element inserts at a new 8bp sequence, it is expected to duplicate this too. Normally this duplication is not seen because the two duplicates go to different gametes. In this case, however, the two go to the same gamete. Yasmine confirmed by sequencing that all the recombinants of this type have an 8bp duplication. Due to the form of the insertion, one of the 8bp sequences is complementary to the original, shown in green compared to red for the original sequence.

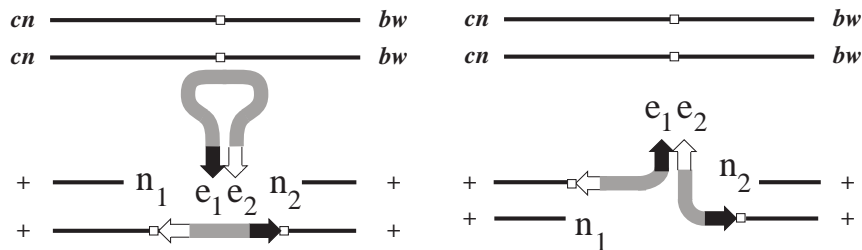


3.8. Hybrid elements formed by sister P elements.

The hybrid elements formed from end-deleted elements are useful in showing unequivocally the nature of the end association. Ultimately what we wanted to show, however, is that normal P elements can also form hybrid elements and thus induce recombination. This work was carried out by Christine Preston and Bill Engels. The expected form of association in this case, again compared to what an element is normally supposed to do, is:

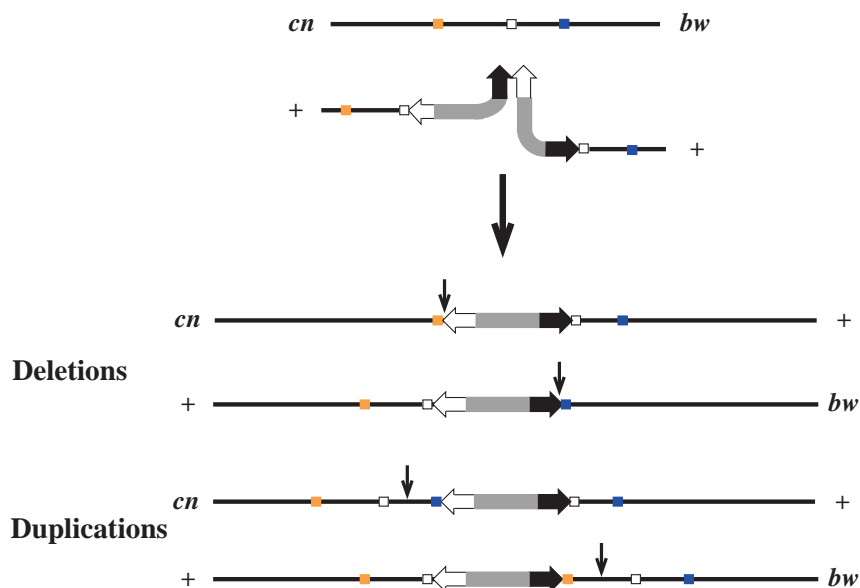


These again resolve by 'excision' to give:



Only insertion recombinants are expected in this case. Repair recombinants are expected to be undetectable sister-strand recombinants.

Depending on the insertion site, recombinants are expected to show either deletions or insertions:



Bill Engels and Christine Preston suggested this as a method for producing knockout mutants. It was, in fact, already known that P elements tend to delete regions around them. In retrospect it seems likely that this is the mechanism whereby such deletions were produced. Their frequency can be increased many times by using recombinant products, targeting specific recombinants from knowing whether the P element lies to the left or the right of the gene whose function one wants to delete. I believe that the method has enjoyed a reasonable amount of success.

3.9. The use of closely linked RFLP markers.

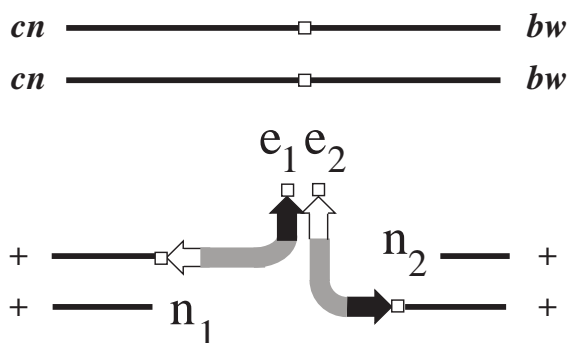
The nature of recombinants can be seen to a certain extent using the PCR primers shown previously. However Bill Engels devised a method that allowed recombinant products to be more accurately characterised. He screened a wide range of laboratory strains for RFLP markers defined by using a range of restriction enzymes. One strain, originating from Japan, was found to have 12 identifiable differences from the chromosome containing *Ca1* [17]. The actual recombination point could thus be reasonably accurately characterised in hybrids involving this strain.

Subsequently we were able to jump our end-deleted elements onto this strain and thus use the same method to characterise recombination

points in this way. We found that nearly 50% of our 'insertion' lines had neither duplications nor deletions. Results from crosses using the RFLP markers were in agreement with a model in which the P element ends did not actually insert, but were instead degraded and repaired, just like the non-P ends [15].

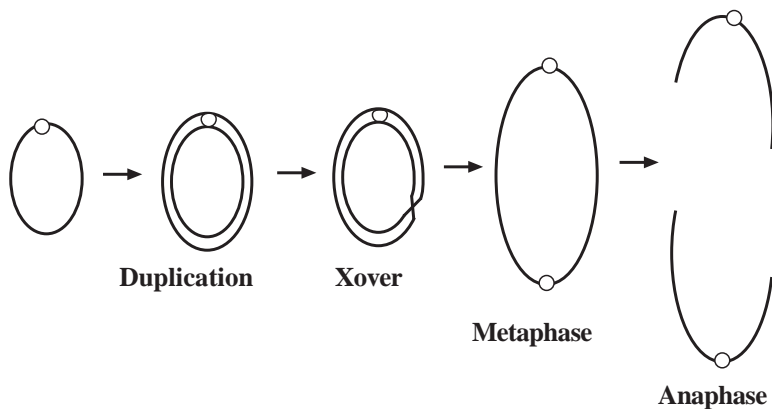
3.10. Sister-strand crossingover and ring chromosomes.

The expected situation when a hybrid element excises is, as shown previously:



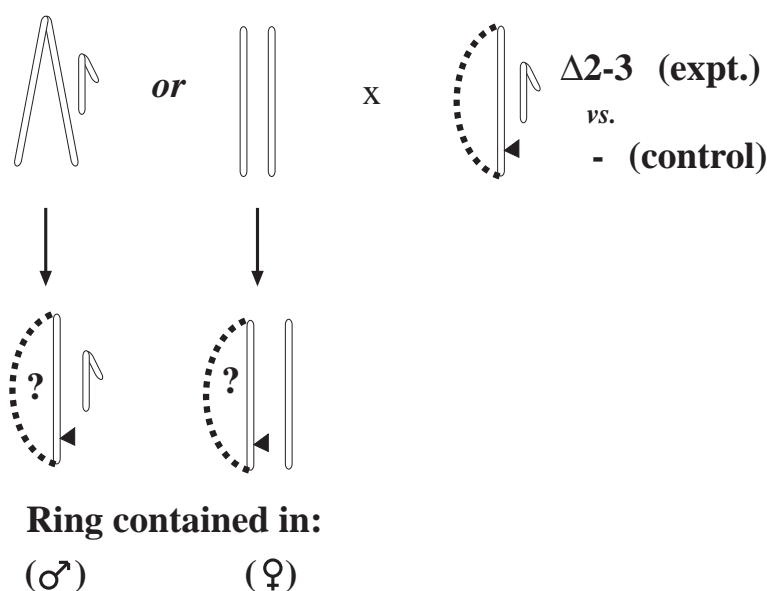
Ends n_1 and n_2 can only join by producing a sister-strand crossover. Ends e_1 and e_2 can produce either sister-strand or non-sister-strand crossingover. Either way, sister-strand crossingover is to be expected from such a structure.

While sister-strand crossingover is not normally expected to have genetic consequences, ring chromosomes provide an exception. Crossingover in such chromosomes, either in mitosis or meiosis, is expected to produce a double ring that can only be resolved by breakage, leading to inviable products.



In the case of ring-X chromosomes, crossingover in the male is expected to lead to inviable X chromosomes. Y chromosomes are unaffected. In the offspring, such X chromosomes are combined with a normal X chromosomes, leading to a potential decrease in female progeny.

Drosophila has another trick stock, in which X chromosomes in the female are attached to each other. Gametes from such a female are either attached X chromosomes or a Y chromosome. In contrast to the normal cross, X chromosomes in crosses to attached-X females go to male progeny.



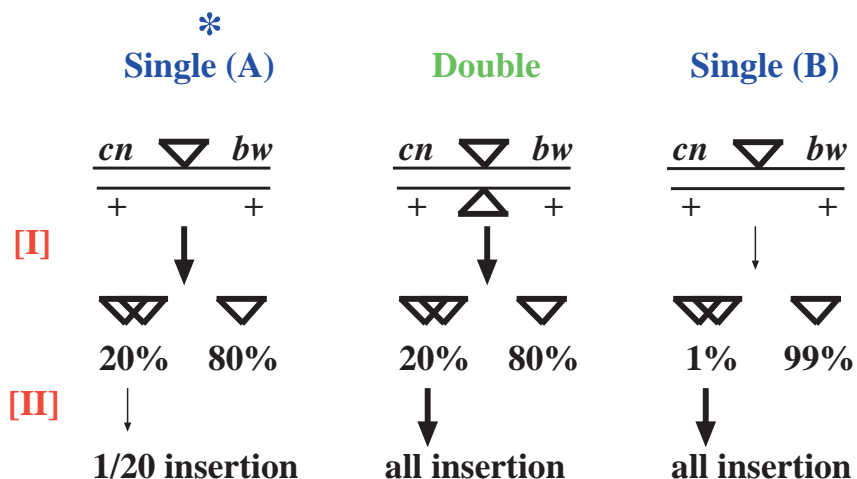
Two different strains were made in which an X chromosome CaSpeR element was recombined onto a ring-X chromosome. The experiment was carried out twice. Results were not entirely consistent, but overall there was at least 20% deficiency of the ring chromosome when inherited by male offspring, indicating a potential 20% sister-strand crossingover [27].

There was, however, little or no deficiency in female progeny. We rationalised this by saying that the extra X chromosome covers up any deletion effects. Years later I realised that we should have extended the experiment by crossing such female progeny to look for deficiencies in their male progeny. Unfortunately the experiment was carried out, and the stocks discarded, a long time before the analysis and publication. We attempted to reconstruct the stocks but were unable to even obtain another ring-X chromosome strain in which the ring had not broken

down. The sad truth is that to my knowledge ours is the only published ring-X *Drosophila* experiment in the last decade or two. Larry Sandler, from whose lab I received the ring-X stock, is no longer alive. I originally learned about ring chromosome theory in undergraduate classes, but in the current molecular era this seems to be one of the old-fashioned techniques that has fallen by the wayside.

3.11. How commonly do hybrid elements occur?

As previously mentioned, single elements induce recombination at the rate of 1% or less. However homologous elements induce it at a rate of around 20%. There are two possible explanations for this difference. Either (A) hybrid elements form at much the same rate when there are single or homologous elements, with a lower rate of insertion in the single case, or (B) they form at a much higher rate when there are homologous elements. At the risk of overcomplicating, the two hypotheses are shown below. For the case of double elements, shown in the middle, both stage [I], formation of double elements and stage [II], insertion to form recombinants, occur at high frequency. For the single element, either stage [I] or stage [II] must be reduced in frequency.



It seems difficult to see why hybrid elements should form 20x more frequently when they are present in two copies. It is true that end-deleted elements on homologous chromosomes can associate. But in that case there is no alternative of sister-strand association. Normally, sister elements might be expected to associate more frequently than non-sisters, although it is not known what proportion of the hybrid elements in the homologous element case are non-sisters.

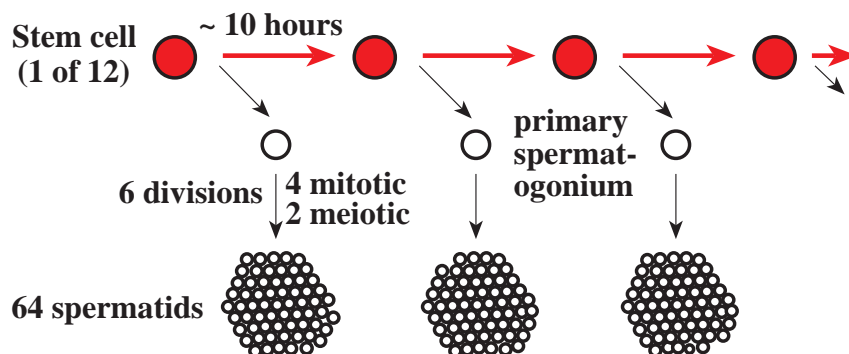
Why, then, should the rate of insertion into the homologue to produce recombination be so much lower for single elements? One strong possibility is that there is no P element on the homologue. P elements are known to have a strong tendency to insert near other P elements, which could explain the much higher recombination frequency in the homologous element case.

If these arguments are accepted, hybrid elements must form much more frequently than indicated by male recombination frequencies, ie. closer to 20% than 1%, in agreement with the results from the ring-X experiment. It's not clear to me why the formation of hybrid elements should be of value to either the transposon or to the fly. It seems hard to believe that the transposon would not have evolved some mechanism to ensure that it associates the correct ends in almost all cases.

3.12. Male recombination occurs in pre-meiotic germline cells.

I haven't said anything about the timing of recombination. In fact it has been known since Hiraizumi's first report [11] that most of the recombination must be mitotic rather than meiotic. Recombinants, when they occur, tend to come in batches, a sure sign that they occur in the germline well before meiosis.

These clusters of recombinants are of two types. Either reciprocal recombinants are present at approximately equal frequency, or else one type is present at much higher frequency. I attempted to take the argument further [29] by analysing the relative frequencies of the two types on the hypothesis that they occur in mitosis at the four strand stage.



The usually accepted picture of *Drosophila* male germline development is shown here. Although primary spermatogonium cells can give rise to

64 sperm, it is likely that the largest clusters come from recombinant chromosomes in stem cells. When a stem cell contains a genotype such as $cn + / + bw$, for example, a batch of reciprocal recombinants is expected. A $cn bw / + bw$ cell leads to a single cluster.

In the paper, I gave the expectation for single : reciprocal clusters as 1 : 1. I got it wrong. The correct expectation should be 2 : 1. The observed numbers in a summary of our combined data was 9 : 11, which I stated to be in agreement with a 1 : 1 ratio. The numbers are sufficiently low, however, to be also in agreement with the 2 : 1 expectation. The data still wouldn't rule out a mitotic crossingover model.

The other conclusion from the paper was that lethal mutations occurred as often in recombinant chromosomes as in non-recombinants. This argues against a model in which recombinants are due to chromosome breakage, which also causes lethality. The hybrid element recombination model provides a partial vindication of this early claim that recombination is close to a regular crossingover event.

The sequence of events in male germline cells was taken further by Mark Tanaka, who studied the course of recombinants in crosses in flies carrying the end-deleted elements as they age [31]. The clusters got more and more extreme as the flies aged, as predicted if stem cells were gradually dying off. Sterility occurred at an earlier age in these flies than in non-dysgenic ones, suggesting that crossingover events were contributing to the rate of stem cell death.

The recombination frequencies increased on average. Some extreme cases of 100% recombination were found. Mark modeled what is expected at particular ages for given rates of recombination, cell division and cell death. Overall I think it is the best theoretical and practical genetical study that has been done on the dynamics of the male germline.

3.13. More on recombination.

I've left out a lot of the details of this saga, which overall went on for more than 30 years. For many of those years after P elements were discovered I believed that we would find that the recombination was due to elements jumping out, leaving broken ends which might then invade and, along with repair processes, be responsible for causing recombination. When we first found the hybrid element effect I initially thought that it might be just a small part of the story. It was my

colleague Yasmine Gray who eventually persuaded me that it really was *the* story.

I haven't mentioned a substantial study by Xiumei Liang looking at all possible combinations of end-deleted and normal elements [16]. The hybrid element insertion and repair model satisfactorily explains all the PCR products and recombination values. I've briefly mentioned her other study [15] in which she showed that P element ends leads to recombination by repair as often as insertion.

In another early aspect of the story, it was claimed that male recombination could be induced by grinding up P stock flies [20], injecting them into laboratory M females which were mated to M wild type laboratory flies. Male progeny were then claimed to induce recombination at a low rate, with zero recombination in the controls. Margaret Kidwell and I were both unable to find a difference between injected lines and controls, and our negative paper was accepted for publication [28]. Subsequently Rubin and Spradling showed that P elements could be injected into embryos, making it seem feasible that the adult injection experiment could have worked. But there is a lot of difference between injection into an egg of a P element-containing plasmid under tightly controlled conditions versus injecting adults with a homogenised extract of flies.

4. SOME CONCLUDING THOUGHTS

4.1. The naming of hybrid dysgenesis.

The hybrid dysgenesis name that Margaret Kidwell and I introduced has been substantially accepted in the literature. I do have some qualms, however, about its applicability given what we now know about its cause.

In the P-M system and the I-R system, and probably also the *hobo* system that hasn't been discussed here, the cause of dysgenesis is specifically the activation of a specific transposon. When we introduced the term I thought of it as some sort of extension of 'hybrid sterility'. But if all cases of this phenomenon are due to specific transposons, I suspect it would be more accurate to use a name such as 'transposon eruption'. This would not bring out the hybrid nature of the phenomenon, so maybe it is not the worst example of inappropriate naming.

4.2. Dysgenesis and spatial organisation of chromosomes.

I couldn't resist writing something about this, although it is probably not a topic that has anything to do with the current chapter.

A lot is now known about the control of chromosome movement by microtubules, and many motor proteins have been identified. But as far as I know there is still no answer to the question of how this organisation is established after fertilisation. In the old days there used to be talk about 'long distance forces of attraction', but I don't believe any such things have been found.

I thought about chromosome organisation as a PhD student, and even had a paper published about possible attachment of telomeres to nuclear membranes to explain the frequency of different sorts of meiotic configurations in tetraploid plants [22]. So maybe this gives me some sort of license to write about such things. Anyway when hybrid dysgenesis came along I immediately thought, based on no evidence at all, about the possibility of spatial organisation as a potential explanation [24].

At fertilisation, the male gamete contributes little other than chromosomes. Any membranes and other spatial organisation units must come from the female parent. Although there is no necessity that these be maternally inherited, there is clearly ample opportunity for non-reciprocal effects. And if the chromosome set has in some way diverged to be no longer compatible with the inherited membranes, then genetical problems might be expected. I have to admit that the phenomenon of chromosomal contamination, the movement of properties from one chromosome to another, was already known for the I-R system, and it took a little mental gymnastics to fit this in with the spatial organisation hypothesis.

Anyway I was so infatuated by this hypothesis that I was the last person in the field to accept that the cause of dysgenesis was transposons of some sort. I finally gave up when the P element was isolated and sequenced. Admitting this cost me payment of a dinner with Michael Ashburner, the *Drosophila* guru at Cambridge University. We had a bet on the cause of dysgenesis. He had bet that the cause would turn out to be some sort of virus, which I had to admit was much closer to the truth than spatial organisation.

4.3. Spatial organisation of chromosomes.

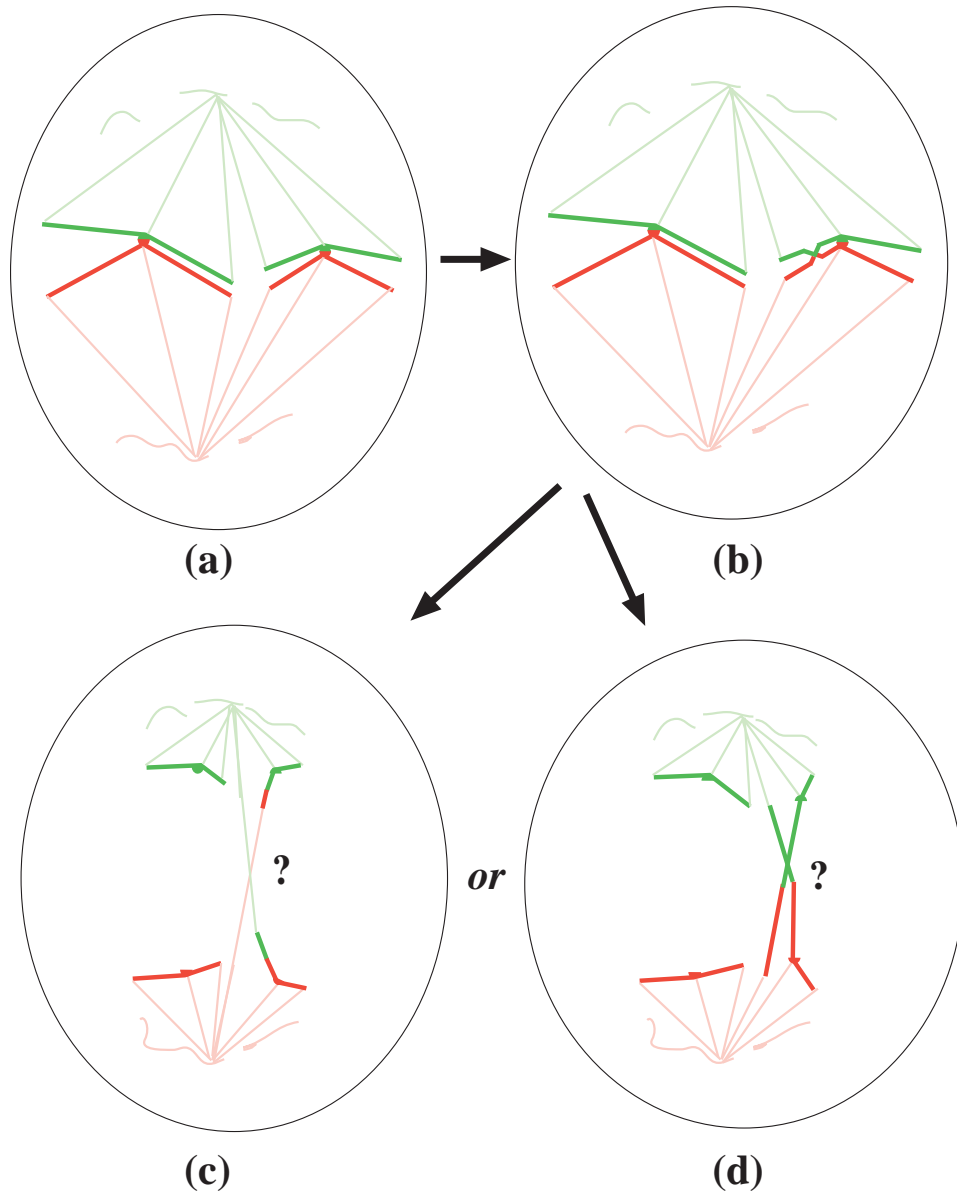


FIGURE 4. Artist's impression of what might happen with sister chromatid exchange (SCE) under an extreme chromosome organisation hypothesis

Although it may have nothing to do with P elements, I still ponder about the inheritance of spatial organisation. The mechanisms responsible for the merging of the maternal and paternal chromosome

set much be under genetical control. Admittedly there must a considerable amount of flexibility in the system to allow for variation in chromosome structure, as exemplified by such examples as the closely related muntjak species where the chromosome numbers range between 6 and 46.

As a believer in underlying order, I have always been attracted to the 'Lark hypothesis'. This postulates an extreme form of spatial organisation of chromosomes, a unified chromosome set, where the chromosomes segregate together in division. Each chromosome contains one new and one old strand of DNA, but one of the old strands will be one generation old and one will be more than one generation old. Karl Lark claimed that all chromatids with the 'newer' one-generation old strand segregate together. We know that at meiosis the chromosomes segregate independently. Under the Lark hypothesis one has to postulate a specific mechanism for ensuring that maternally and paternally derived chromosomes segregate independently in meiosis, rather than relying on the idea that chromosomes are separate and that this independence happens naturally.

The Lark hypothesis is closely related to the 'immortal DNA strand' hypothesis of John Cairns, in which the original strand of DNA is directed to particular tissues, eg the germline, to minimise the effects of mutation. This also implies a control of the overall chromosome set. Someone has written an interesting account of this on Wikipedia (http://en.wikipedia.org/wiki/Immortal_DNA_strand_hypothesis)

One aspect of these hypotheses that intrigues me is the thought of what happens with sister chromatid exchange. SCEs usually seem to be looked on as passive markers of chromosome instability - after all they are just exchanging identical segments. But could they be a cause of instability beyond just the possibility of some inequality in the exchange?

The diagram above shows a picture of a cell after chromosomes have divided. The 'newer' DNA chromosomes are pictured as green and the 'older' ones as red. The cell must be organised somehow so that the green chromosomes go to one pole and the red to the other. I have pictured this by colouring the microtubules and membranes appropriately, although the colours in this case refer to their organisation rather than to the DNA age distinction.

The point of this is to show that there could be a problem if SCE occurs - basically 'newer' material (green) gets connected to 'older' material

(red). What happens then? Does this lead to problems? This might be either with the extrachromosomal material (c) or the chromosomes themselves (d). Either way, something has got to give.

It is not clear, in fact, how often SCEs occur in normal cells. SCE happens in cultured cells, but these are usually highly abnormal, with a tendency for chromosomal complement to break down. I don't think anyone would believe in the highly stylised organisation implied by the diagram or in the Lark hypothesis in its extreme form, but there are intermediate forms that may have some of the same properties. Otherwise it is difficult to see why SCEs should have negative consequences for the survival of the cell. I assume that people must have thought about this but I haven't seen anything.

Returning to P elements, a series of impressive papers by Ronsseray and collaborators[12] indicates that the primary source of P element repression comes from elements inserted in heterochromatic regions close to the telomeres, apparently of any chromosome. Insertion into centromeric heterochromatin doesn't work. Furthermore it takes several generations for the repression to reach its maximum value. The effects seem to be mediated via small RNA molecules. But none of this really clarifies the cytoplasmic component. Perhaps spatial organisation will turn out to have something to do with this, even though it spectacularly failed to be the primary cause of the dysgenesis.

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