HYBRID DYSGENESIS IN *DROSOPHILA* MELANOGASTER: THE FREQUENCY AND DISTRIBUTION OF MALE RECOMBINATION EVENTS

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SUMMARY

The frequency and distribution of male recombination events induced in dysgenic hybrids of *Drosophila melanogaster* has been investigated. Two wild-type inbred lines extracted from the "Texas" population were shown to differ substantially in the location of chromosome breakage points, thereby providing evidence that populations display heterogeneity of integration sites for the transposable elements responsible for dysgenic interactions. An attempt has been made to relate these data to current views on the molecular basis of hybrid dysgenesis.

Evidence is provided which suggests that male recombination, SF female sterility and GD female sterility are induced by the same mobile genetic elements, indicating an identity of the P–M and I–R interaction systems. The basis of the reported independence of these systems is discussed.

1. INTRODUCTION

In the past decade there has been considerable interest in various genetic aberrations found in hybrids from certain wide crosses of *Drosophila melanogaster*. The most obvious aberrations are varying degrees of male and female sterility, both in terms of egg and sperm production, known as gonadal dysgenesis or GD sterility (Kidwell *et al.*, 1977) and reduced hatchability of eggs, termed SF sterility (Picard *et al.*, 1977). Other aberrations include male recombination, increased mutation rates, meiotic drive and a generally high frequency of chromosomal abnormalities, (Hiraizumi *et al.*, 1973; Waddle and Oster, 1974; Matthews and Hiraizumi, 1978; Woodruff and Thompson, 1977; Sved, 1974; Slatko and Hiraizumi, 1973; Cardellino and Mukai, 1975; Yamaguchi, 1976; Voelker, 1974; Hiraizumi, 1971; Henderson *et al.*, 1978; Kidwell and Kidwell, 1975).

The traits are known to arise from a nuclear-cytoplasmic interaction and, as far as they have been studied (Kidwell, 1979), the controlling mechanisms of the various aberrations are essentially similar. The term "hybrid dysgenesis" has been proposed to refer collectively to this syndrome of abnormalities (Sved, 1976).

It has been suggested (Kidwell, 1979) that there are two independent systems of interaction within the hybrid dysgenesis syndrome. First, there is the I–R system which is characterised by SF sterility, increased mutation rates and non-disjunction but in which dysgenic male effects are reportedly absent (Bregliano *et al.*, 1980). Secondly, there is the P–M system which is characterised by GD sterility and also by the occurrence of male recombination (Kidwell, 1979). The two systems, however, show marked similarities. Both involve the existence of two types of strain whose interaction leads to any of a number of dysgenic traits. The first type of strain carries a

cytoplasmic factor, thought to be a population of cytoplasmic particles and is restricted in the main to long established laboratory stocks. Strains of this type have been described within the I-R system as either reactive (R), if their use as female parents results in the production of dysgenic progeny, or neutral (N) if it does not. Within the P-M system strains of this type are described respectively as maternally contributing (M), or neutral (Q). There is some doubt as to whether truly neutral strains exist due to the fact that reactivity displays marked quantitative variation. It may be that they represent a very weak cytoplasmic effect. In both systems the cytoplasmic state is ultimately controlled by the chromosomal genotype (Kidwell, 1979; Eggleston, 1981).

The second type of strain carries a chromosomal factor or factors which may be linked to any or all of the major chromosomes and possibly to the fourth. Strains of this type are described as inducer (I) within the I-R system, or paternally contributing (P) within the P-M system. It has been suggested for both systems that this chromosomal factor may be a transposable element (Bregliano *et al.*, 1980) and recent evidence would support this (Rubin *et al.*, 1982; Bingham *et al.*, 1982). Within each of the systems any cross between a female carrying the cytoplasmic factor and a male carrying the chromosomal factor (Cross A) results in F1 progeny which display a range of dysgenic traits. The reciprocal cross (Cross B) leads to the production of normal progeny.

Recent work has indicated that the description of two independent systems may be an artefact of the temperature sensitivity of the dysgenic traits. Both SF and GD female sterility have been shown to have a common causation and a very similar increase in expression with rising developmental temperature (Eggleston and Kearsey, 1980; Eggleston, 1981). There is also evidence that other dysgenic traits, notably male recombination, respond to developmental temperature in much the same way (Yannopoulos and Pelecanos, 1977).

An attempt has been made in this paper to show that male recombination, SF sterility and GD sterility are induced by the same dysgenic interaction and that the description of independent P-M and I-R systems (Kidwell, 1979) is merely an artefact of the measurement techniques used. The frequency and distribution of male recombination events has been investigated in various crosses and an attempt made to relate these data to current views on the molecular basis of hybrid dysgenesis.

2. MATERIALS AND METHODS

(i) *Strains used*

(a) *Marker stocks*

ru	st	ss	ca				
ru	h	th	st	cu	sr	e ^s	ca

These two strains each carry a series of recessive markers on the third chromosome. The markers are described fully by Lindsley and Grell (1968). Both strains are known to possess strongly reactive cytoplasmic factors and to be devoid of the chromosomal inducing factors.

(b) *Potential inducer strains*

TEX 1

TEX 6

These are two wild-type inbred lines derived from the "Texas" population. This population was established in 1965 from inseminated females captured at Austin, Texas and the inbred lines have been maintained by sib-mating for more than 200 generations prior to their use in these experiments (Linney *et al.*, 1971).

(ii) *Measurement of female sterility*

All measurements of egg production (GD sterility) and egg hatchability (SF sterility) were carried out as described by Eggleston and Kearsey (1980).

3. THE RELATIONSHIP BETWEEN MALE RECOMBINATION AND FEMALE STERILITY

Two wild-type inbred lines known to possess third chromosome factors capable of inducing both SF and GD sterility were investigated with respect to their ability to induce male recombination. The two inbred lines, TEX 1 and TEX 6 were crossed reciprocally to *ru st ss ca*. For each cross, the production and hatchability of eggs from the female F1 progeny were assessed in order to confirm the presence of both SF and GD sterility in Cross A. In this way, any male recombination found could be compared directly to known female sterility patterns among the same progeny. Hatchability and egg production measurements were transformed to improve the normality of their distributions. To this end, percentage hatchabilities were transformed to angles with values of 0 per cent and 100 per cent being increased and decreased respectively by $1/(4N)$, following Bartlett (1947). In every case N was 25, this being the number of eggs sampled in order to calculate the hatching percentage. Examination of the egg production data revealed a high correlation between the mean and the variance ($r = 0.6$) and the appropriate transformation in this case is a conversion to square roots. Each egg production measurement was therefore adjusted accordingly.

In order to ascertain the levels of male recombination induced by each inbred line, 50 F1 males from each of Cross A and Cross B were individually backcrossed to 10 marker females. Upon eclosion the progeny were scored for the presence of recombinants in the *st-ss* and *ss-ca* regions. The *ru-st* region was not used because of the poor expression of roughoid (*ru*) in this stock.

The results of the sterility check (table 1(a)) confirm the presence of both SF and GD sterility in Cross A from each of TEX 1 and TEX 6. It is evident that both hatchability and egg production are substantially reduced in the dysgenic cross. The two inbred lines induce similar levels of SF sterility although the depression of egg production (GD sterility) is somewhat stronger for TEX 6.

The results of the male recombination measurements are summarised in table 2 and they show quite clearly that;

(a) Spontaneous male recombination has occurred in the progeny of Cross A for both TEX 1 and TEX 6.

TABLE 1

Mean SF sterility (percentage egg hatchability transformed to angles) and mean GD sterility (number of eggs laid per female per hour transformed to square roots) for TEX 1 and TEX 6 when crossed reciprocally to the marker stocks; (a) ru st ss ca and (b) ru h th st cu sr e⁺ ca at 25°C to form Cross A and Cross B

		(a)		(b)	
		TEX 1	TEX 6	TEX 1	TEX 6
SF	Cross A	7.62 ± 2.77	10.21 ± 4.79	33.09 ± 14.87	33.43 ± 6.63
	Cross B	78.54 ± 5.88	77.63 ± 6.14	80.49 ± 4.75	82.03 ± 3.83
GD	Cross A	1.21 ± 0.20	0.71 ± 0.23	1.25 ± 0.23	0.97 ± 0.20
	Cross B	1.40 ± 0.32	1.41 ± 0.23	1.65 ± 0.22	1.80 ± 0.15

TABLE 2

Numbers of parental and recombinant progeny types and percentage male recombination frequencies from TEX 1 and TEX 6 when crossed reciprocally to ru st ss ca at 25°C, forming Cross A and Cross B

			Cross A		Cross B	
Progeny genotype			TEX 1	TEX 6	TEX 1	TEX 6
+	+	+	1640	392	1403	1334
st	ss	ca	1711	384	958	854
st	+	+	21	9	0	0
+	ss	ca	23	3	0	0
st	ss	+	12	5	0	0
+	+	ca	8	2	0	0
st	+	ca	1	0	0	0
+	ss	+	3	0	0	0
Total progeny			3419	795	2361	2188
Total recombination events			72	19	0	0
Recombination frequency			2.11	2.39	—	—
Recombination events (st-ss)			48	12	—	—
Recombination freq. (st-ss)			1.40	1.51	—	—
Recombination events (ss-ca)			24	7	—	—
Recombination freq. (ss-ca)			0.70	0.88	—	—

(b) The male recombination frequencies induced by TEX 1 and TEX 6 are very similar at 2.1 per cent and 2.4 per cent respectively.

(c) Male recombination is absent in the Cross B progeny and thereby displays the reciprocal cross effect characteristic of dysgenic traits.

Thus, two inbred lines from the "Texas" population chosen simply because they were known to possess third chromosome factors capable of inducing similar levels of female sterility, had also been shown to induce similar levels of male recombination, providing further evidence for the correlated induction of dysgenic traits. TEX 1 and TEX 6 not only induced similar levels of male recombination but also yielded similar recombination frequencies between the markers on the right arm of the third chromosome (table 2). These recombination frequencies, however, were very different from the accepted female meiotic map for this chromosome (Lindsley and

Grell 1968). In fact, the proportion of recombinants in the regions st-ss and ss-ca changed from approximately 1:3 in the female to about 2:1 in the male. The presence of the centromeric heterochromatin evidently has little effect on the frequency of male recombination events.

4. THE DISTRIBUTION OF MALE RECOMBINATION EVENTS

In order to make further assessments of the frequency and, in particular, the distribution of male recombination events, a series of larger experiments was designed to survey the whole of the third chromosome and also to examine the inducing potential of isolated wild-type third chromosomes from both TEX 1 and TEX 6. Due to the scale of the experiments a decision was taken to concentrate on the dysgenic cross only. This was felt to be justified since in the preliminary experiments no recombinants had been found among a total of 4549 Cross B progeny (table 2).

Males from TEX 1 and TEX 6 (experiments 1 and 2 respectively) were crossed to ru h th st cu sr e^s ca females forming Cross A. Of the resulting progeny, 90 F1 males were individually backcrossed to 10 marker females for each of the experiments. The progeny were scored for the presence of recombinants between each of the eight markers. As before, the production and hatchability of eggs from the F1 females was assessed to confirm the presence of both SF and GD sterility (table 1(b)). Once again, both egg production and hatchability were found to be substantially reduced in Cross A and as before, whilst there were similar levels of SF sterility the depression of egg production (GD sterility) was greater for TEX 6.

On the evidence of the preliminary experiments, male recombination was expected to occur in the progeny of Cross A because of the presence of inducing factors on the third chromosomes of TEX 1 and TEX 6. However, this simple design exercises no control over the rest of the wild-type genome which may possess further inducing factors capable of interacting with the third chromosome. For this reason two further experiments were designed to eliminate all potential inducing factors other than those on the third chromosome.

This was achieved by crossing each inducing line to a standard y bw st marker stock which possessed neither reactive cytoplasm nor inducing factors. If potential inducing chromosomes are denoted +', then F1 males with the genotype y/7 bw // + ' st // + ' can be backcrossed to the y bw st marker stock and males of the genotype y/7 bw // bw st // + ' isolated from the progeny. These males each carry a unique wild type third chromosome which may possess inducing factors. This chromosome can be paired with a suitably marked homologue by crossing the male to females of the third chromosome marker stock ru h th st cu sr e^s ca. From the progeny, males of the genotype +/7 // bw ru h th st cu sr e^s ca // + ' can be isolated and screened to see if the wild type third chromosome (+') has induced recombination. For each of TEX 1 and TEX 6 (experiments 3 and 4 respectively) 104 males carrying a potentially inducing wild type third chromosome were individually backcrossed to ten ru h th st cu sr e^s ca females forming Cross A. It should be noted that this experimental design also eliminates any possibility of chromosomal contamination (Picard, 1976) since the potential inducing factors are always maintained in the male line and are not activated by a dysgenic interaction.

TABLE 3

Percentage male recombination frequencies from experiment; (1) TEX 1 uncontrolled genetic background, (2) TEX 6 uncontrolled genetic background, (3) TEX 1 controlled genetic background and (4) TEX 6 controlled genetic background

	Experiment number			
	1	2	3	4
Total progeny	8320	9349	11 184	8271
Recombination events	224	242	161	212
Recombination frequency	2.69	2.59	1.44	2.56

Experiments 1 and 2 may be referred to as having an *uncontrolled* genetic background, that is, one in which inducing factors may be present anywhere in the wild-type genome. Experiments 3 and 4 have a *controlled* genetic background in the sense that inducing factors are restricted to the wild-type third chromosome.

The results of these experiments are summarised in table 3. In order to make such a summary, a recombination event was defined as any exchange between the marker chromosome and the wild-type chromosome. To this end, double and treble recombinants were classified as two and three recombination events respectively.

Comparison of the results for TEX 1 and TEX 6 in an uncontrolled genetic background (table 3, experiments 1 and 2) reveals that once again the two inbred lines induce similar male recombination frequencies at 2.69 per cent and 2.59 per cent respectively. These figures are comparable with those from the preliminary experiments (table 2) of 2.11 per cent and 2.39 per cent respectively, where the background was also uncontrolled. This is not the case, however, when the genetic background is controlled (table 3, experiments 3 and 4). In the case of TEX 6, controlling the genetic background (compare experiments 2 and 4) produces very little change in the male recombination frequency. For TEX 1, however, (compare experiments 1 and 3) the male recombination frequency is reduced by almost one half (46 per cent). Thus, the elimination of all inducing factors other than those on the third chromosome has a large effect for TEX 1 but comparatively little effect for TEX 6.

A possible interpretation of this anomaly comes from a knowledge of the female sterility characteristics of the two lines. Factors capable of inducing female sterility are present on both the second and the third chromosomes of TEX 1 but only on the third chromosome of TEX 6. If these same factors are responsible for the induction of male recombination then it might be expected that controlling the genetic background of TEX 6 would result in little change to the male recombination frequency. Controlling the genetic background of TEX 1, however, removes the inducing factors known to be present on the second chromosome and therefore the possibility of their interaction with the third chromosome. This could well result in a reduction of male recombination frequency such as we find in the present data. The same effect is seen in the case of SF sterility where controlling the genetic background of TEX 1 reduces the levels of sterility. In fact, egg hatchability was found to rise from 10 per cent to 22 per cent when inducing

factors were limited to the third chromosome (Eggleston, 1981). This increase by a factor of about two is much the same as that observed in the present data for male recombination, thereby providing further evidence for the identity of dysgenic traits and also for the additivity of the inducing effect.

The distribution of male recombination events over the third chromosome may be visualised in two ways. First, by means of the *proportion* of recombination events falling into each chromosome region, which displays the relative positions of the markers on a fixed scale. Secondly, the distribution may be shown in terms of the true recombination frequencies. The proportion of recombination events within each of the seven chromosome regions for experiments 1 to 4 is depicted in fig. 1. Also included for comparison are the comparable maps produced by normal female meiotic recombination, cytological preparations of polytene chromosomes and X-ray induced male recombination (Becker, 1974). Several striking comparisons can be made between these maps. It is evident from fig. 1 that all of the observed male recombination maps (experiments 1 to 4) depart extensively from the accepted female meiotic map. Neither can the observed male recombination maps be seen to agree with the distribution of markers in polytene preparations although there are some similarities in the positioning of the markers on the right arm. The agreement is somewhat closer than in the case of the female meiotic map however, essentially because of the increased map distance between scarlet and curled in the polytene preparations.

The X-ray induced male recombination map perhaps provides the nearest equivalent to the observed results for TEX 1 (fig. 1(d, f)) although agreement with the observed maps for TEX 6 (fig. 1(e, g)) is poor. Bombardment with X-rays is believed to cause random chromosome breakage and the subsequent repair often results in a recombination event. The X-ray induced map should, therefore, be related solely to the physical length of DNA between the markers. Heterochromatic regions, such as those around the centromere, which have a large amount of DNA (albeit in a condensed form) would be expected to show high recombination frequencies after treatment with X-rays. This is clearly shown by the map distance between st and cu in the X-ray induced map (fig. 1(c)). However, any agreement between the observed male recombination maps and the X-ray induced map is weakened by consideration of the left arm markers ru to st. For these regions, TEX 1 induces less and TEX 6 substantially more recombination events than treatment with X-rays. It would seem, therefore, that the observed male recombination is not due merely to random chromosome breakage as in the case of X-ray treatment, but is being controlled in some way by the inducing factors known to be present within the genome.

Consideration of the observed results shows that by far the largest differences are due to the choice of inducing strain. TEX 1 and TEX 6 produce very different distributions of the markers on the left arm of the third chromosome, although the distribution of markers on the right arm is more consistent. Such differences were unexpected since the lines induce very similar absolute recombination frequencies. It would appear that the differences reflect substantial variation in the distribution of inducing factors within populations.

FIG. 1. Map positions of the third chromosome markers based on the proportion of recombination events occurring in each chromosome region for; (a) female meiotic recombination, (b) cytological preparations of polytene chromosomes, (c) X-ray induced male recombination, (d) Experiment 1 (TEX 1 uncontrolled genetic background), (e) Experiment 2 (TEX 6 uncontrolled genetic background), (f) Experiment 3 (TEX 1 controlled genetic background), (g) Experiment 4 (TEX 6 controlled genetic background).

The extremely low frequency of male recombination events in the left arm of the TEX 1 third chromosome (fig. 1(d, f)) is an interesting feature. Initially it was thought that an inversion or other gross chromosomal defect might have been responsible. However, an analysis of the polytene chromosomes showed that no obvious defects were present (unpublished data). Furthermore, the presence of any chromosomal defect would presumably also restrict the number of recombination events recovered from the female. It is clear from table 4 that there is no restriction of recombination events in the left arm regions and this would seem to preclude the presence of an inversion or other gross defect. In fact, one of the left arm regions (h–th) shows a large excess of recombination events when compared with the accepted female meiotic map. The reason for this excess is unclear but it would seem to be unrelated to the hybrid dysgenesis syndrome because it appears to an almost equal extent in both Cross A and Cross B. The excess

TABLE 4

Percentage female recombination frequencies recovered from Cross A and Cross B between TEX 1 and ru h th st cu sr e^s ca at 25°C. The standard female meiotic map (Lindsley and Grell, 1968) is included for comparison

Region	Cross A	Cross B	Standard female meiotic map
ru–h	27.4	32.0	26.5
h–th	29.4	26.3	16.7
th–st	1.4	1.1	0.8
st–cu	7.5	5.7	6.0
cu–sr	6.8	8.6	12.0
sr–e ^s	6.8	2.8	8.7
e ^s –ca	20.5	23.4	30.0

of recombination events in this region is the cause of a significant deviation from the accepted female meiotic map; Cross A, $\chi^2_{(5)} = 16.0$, $P = 1.0$ per cent; Cross B, $\chi^2_{(5)} = 13.1$, $P = 5.1$ per cent. Indeed, if this region is excluded there is scant evidence of any deviation from the accepted female meiotic map; Cross A, $\chi^2_{(4)} = 6.4$, $P = 30$ –10 per cent; Cross B, $\chi^2_{(4)} = 7.6$, $P = 30$ –10 per cent. The point of major importance, however, is that there is no evidence of any chromosomal defect which might limit the frequency of male recombination events observed in the left arm of the TEX 1 third chromosome. Evidently, this lack of male recombination events must reflect in some way the action of the inducing factors within the TEX 1 genome.

It is interesting to note the effects of controlling the genetic background. Clearly this process alters the distribution of the markers and for TEX 1 (fig. 1(d, f)) we find that controlling the genetic background results in fewer recombination events to the left of the centromere and a greater proportion to the right. This pattern is repeated for TEX 6 (fig. 1(e, g)) where the trend is perhaps even more pronounced. These changes in distribution are highly significant for both TEX 1 ($\chi^2_{(4)} = 20.97$, $P < 0.001$) and TEX 6 ($\chi^2_{(5)} = 45.45$, $P < 0.001$). It is, as yet, unclear why there should be a distinction between the left arm of the third chromosome, where we find substantial variation between inducing lines in the map positions of markers and a clear response when controlling the genetic background which we might call "high activity" and the right arm where we find a tendency towards "low activity". It is clear, however, that inducing factors located on non-homologous as well as homologous chromosomes can cause significant changes in the distribution of male recombination events.

Controlling the genetic background also has an effect on the absolute male recombination frequencies. There is a substantial reduction in male recombination frequency for TEX 1 (fig. 2(a, c)) as well as alterations in

FIG. 2. Map positions of the third chromosome markers based on the observed frequency of male recombination events in each chromosome region for; (a) Experiment 1 (TEX 1 uncontrolled genetic background), (b) Experiment 2 (TEX 6 uncontrolled genetic background), (c) Experiment 3 (TEX 1 controlled genetic background), (d) Experiment 4 (TEX 6 controlled genetic background).

the distribution of markers. This pattern is not, however, repeated for TEX 6 (fig. 2(b,d)), where there is only a change in the distribution of markers. In the case of TEX 1, the changes are thought to be due to the elimination of inducing factors known to be present on the second chromosome. The changes observed for TEX 6 would seem to indicate that inducing factors must be present within the genome at sites other than the third chromosome. From the data for TEX 1, it is apparent that the involvement of another large chromosome (in this case the second) tends to cause dramatic changes in male recombination frequency. Female sterility studies (Eggleston, 1981) have shown that the second chromosome of TEX 6 is not involved to any measurable extent in the induction of dysgenic traits and all of the crossing schemes used in the male recombination experiments exclude any potentially inducing X chromosomes. This does not, however, preclude the involvement of inducing factors on the Y or fourth chromosomes in any interaction with chromosome 3.

Controlling the genetic background was also found to reduce the frequency of multiple male recombination events. In the case of TEX 1, the frequency of double recombinants fell by 35 per cent from 2.68 per cent to 1.86 per cent. This could, perhaps, be explained by the overall reduction in male recombination frequency observed for TEX 1 upon controlling the genetic background except that a similar trend is seen for TEX 6 where no such reduction in frequency occurs. The frequency of double recombination events induced by TEX 6 fell by 26 per cent from 15.29 per cent to 11.32 per cent when the genetic background was controlled and the frequency of treble recombination events fell from 2.07 per cent to zero. These reductions and the other changes seen upon controlling the genetic background are consistent with the elimination of inducing factors on non-homologous chromosomes and provide clear evidence of transposition between chromosomes.

5. DISCUSSION

The experiments presented in this paper describe the induction of certain hybrid dysgenic traits by two wild-type inbred lines extracted from the same natural population. The two inducing lines, TEX 1 and TEX 6, were chosen simply because they induced similar levels of each type of female sterility when crossed to reactive strains. The fact that they were also found to induce similar levels of male recombination would seem to provide evidence that the same inducing factors (located within the wild-type genome) were responsible for the induction of all three dysgenic traits.

Recent arguments that the induction of hybrid dysgenesis is determined by two independent systems are based partly on evidence that the distribution of strain types differs between the I-R and P-M interactions. It has been reported, for example, that I strains may be either M, P or Q in the P-M system and that M strains may be I in the I-R system (Kidwell, 1979). This same report however, does conclude that "most strains have the potential for both types of interaction". Clearly the evidence for independence depends to a large extent upon the original classification of strains as I, R, N, P, M or Q. This, in turn, is determined from a strain's ability to induce dysgenic traits or, more specifically, SF and GD female sterility. It is here that we find certain anomalies in the literature.

The characteristics of the I-R interaction are well documented and the principle trait of this system, namely SF sterility, is accepted and treated as a continuous variable. Furthermore, it is known that the expression of this character is dependent on the developmental temperature of the dysgenic cross. Within the normal limits an increase in developmental temperature will lead to an increase in the level of SF sterility (Eggleston and Kearsey, 1980). There is an anomaly, however, in the treatment of the principle trait within the P-M system, namely gonadal dysgenesis or GD sterility (Kidwell *et al.*, 1977). Egg production, which is the parameter of interest in this trait, is also a continuous variable but is not treated as such in the description of the P-M interaction (Kidwell, 1979; Engels 1979). These reports suggest that a female is GD sterile if she lays no eggs but normal if she lays one or more eggs which introduces a clear bias and conceals the range of variation displayed by this character in dysgenic crosses. A similar problem is encountered with the work of Stamatis *et al.*, (1981) where the same character is measured by the degree of ovarian atrophy. Although the authors clearly found quantitative variation they introduced three arbitrary and discontinuous classes in to this character, namely complete atrophy (A), reduced ovaries (R) and normal ovaries (N). In common with the work of Kidwell (1979), however, they considered a female to be GD sterile only if she had complete bilateral atrophy of the ovaries and could lay no eggs.

A further technical anomaly results from the response of these characters to developmental temperature. In distinguishing the P-M and I-R systems Kidwell (1979) and Engels (1979) both regularly assess the levels of SF sterility at developmental temperatures of 20°C or below. Their method for the measurement of GD sterility, however, involves a developmental temperature of 29°C. Under such conditions only extreme SF sterility effects would be detected and many crosses would appear normal. On the other hand, it is likely that most dysgenic crosses would display GD sterility due to the use of a high developmental temperature. In this way a cross could easily appear to display GD sterility but no SF sterility leading to a proposal of independence for the two systems. In fact, it has been shown that by appropriate manipulation of strains and developmental temperatures, all possible combinations of SF and GD sterility can be produced (Eggleston, 1981). Clearly, at a developmental temperature of 29°C egg production in the dysgenic cross is minimised and the extreme of the distribution, namely the complete absence of eggs, provides a convenient and discontinuous variable which would respond over a narrow critical range of developmental temperatures as reported by Kidwell (1979). In fact, surveys of more than 20 strains available in this laboratory have shown that GD sterility, when not subjected to such extremes of measurement, displays a progressive increase with developmental temperature as do the other dysgenic traits (Eggleston and Kearsey, 1980; Eggleston, 1981). To date no cross has been found in this laboratory which displays one form of female sterility to the exclusion of the other. Indeed, if both SF and GD sterility are treated as the continuous variables which they clearly are, there is scant evidence for their independence. Further evidence for this identity of dysgenic traits is provided by several wild-type strains, including TEX 1 and TEX 6, which have been shown to induce not only female sterility and male recombination, but also high mutation rates and meiotic drive (Bryan, 1979). It would

appear that such strains have the capacity to induce all dysgenic traits when given suitable conditions.

The capacity to induce significant levels of male recombination has been demonstrated for both TEX 1 and TEX 6 when crossed to marker stocks carrying a reactive cytoplasm. The two lines were found to induce similar absolute male recombination frequencies in the third chromosome but to differ in the distribution of the recombination events. In the case of TEX 6 male recombination events were distributed relatively evenly whereas for TEX 1 the events were localised to the centromeric (st-cu) region. In some respects, TEX 1 is more typical of previous reports (Kidwell *et al.*, 1976) which indicate high frequencies of male recombination around the centromere. The data presented here for TEX 6, however, show that this pattern is not universal.

A notable difference between the two inbred lines was that TEX 1 induced very few male recombination events in the left arm of the third chromosome. This is an interesting phenomenon and there have been several previous reports of lower dysgenic activity in the left arm regions of certain autosomes with respect to both male recombination and mutation induction (Kidwell and Kidwell, 1975; Kidwell and Kidwell, 1976; Woodruff and Thompson, 1977; Minamori and Ito, 1971; Voelker, 1974). In the case of TEX 1 this scarcity of male recombination events cannot be explained by the presence of inversions or other gross chromosomal defects as these have been excluded by investigations of both female recombination and polytene preparations. It is more likely that this reduced activity is related in some way to the distribution of inducing factors throughout the wild-type genome. The observed differences between TEX 1 and TEX 6 make it clear that there is variation in the distribution of these factors within the Texas population.

Many recent reports on the hybrid dysgenesis syndrome have concluded that the inducing factors within the wild-type genome must be mobile genetic elements with a variety of possible integration sites. The system therefore provides an ideal opportunity for the detection and analysis of eucaryotic transposable elements and recently there have been significant advances in this approach (Rubin *et al.*, 1982; Bingham *et al.*, 1982; Ish-Horowicz, 1982). It appears that the transposable elements responsible for the induction of hybrid dysgenesis are generally present in wild-type strains at a frequency of 30–50 elements per haploid genome but are generally absent from strains which carry a reactive cytoplasm (Rubin *et al.*, 1982). The same authors found direct evidence for the “chromosomal contamination” reported by Picard (1976) in that inducing elements were observed to transpose to the chromosomes of a reactive strain previously lacking these elements, thereby rendering them capable of acting as inducing chromosomes themselves. The elements were only mobilised by the dysgenic interaction, that is in the presence of a reactive cytoplasm, a fact which had long been appreciated from the genetic studies of Picard and others.

It is now reasonable to assume that all dysgenic traits so far observed could readily be accounted for by the integration and excision of these mobile genetic elements. For example, male recombination is likely to be due to chromosome breakage and reunion following an excision process. Recent evidence suggests that the integration sites of these elements are not distributed at random throughout the genome but have certain preferred

locations (Engels *et al.*, 1981; Rubin *et al.*, 1982). Engels referred to these points of frequent chromosome breakage as "hot spots". This new evidence may well account for the differences in distribution of male recombination events reported in this paper for TEX 1 and TEX 6. Thus, for example, it is clear that there are very few integration sites or "hot spots" in the left arm of the TEX 1 third chromosome. This results in few chromosome breaks and very low levels of male recombination. The very mobility of these elements provides a simple and adequate explanation for the effects observed on non-homologous chromosomes and for the additive effects of chromosomes bearing inducing factors. Furthermore, the confirmation of the existence of such elements may help to clarify the nature of cytoplasmic reactivity. Bingham *et al.*, (1982) observed a discrepancy in the insertion and excision rates of these elements in that there was an excess of insertions. This excess may be due to the insertion of extra-chromosomal elements when reactive and inducer strains are crossed. Such an eventuality would support the contention that reactivity is determined by a population of cytoplasmic particles, in this case a population of mobile genetic elements.

Clearly there is still much to be learned from an evaluation of the molecular basis of hybrid dysgenesis. Hopefully the information gained from genetic studies will complement the molecular analysis and perhaps yield further information as to the evolutionary significance of this dynamic system.

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