Somatic Effects of *P* Element Activity in *Drosophila melanogaster*: Pupal Lethality

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ABSTRACT

Nonautonomous *P* elements normally excise and transpose only when a source of transposase is supplied, and only in the germline. The germline specificity depends on one of the introns of the transposase gene which is not spliced in somatic cells. To study the effects of somatic P activity, a modified *P* element (Δ2-3) lacking this intron was used as a source of transposase. Nonautonomous *P* elements from a strain called Birmingham, when mobilized in somatic cells by Δ2-3, were found to cause lethality, although neither component was lethal by itself. The three major Birmingham chromosomes acted approximately independently in producing the lethal effect. This lethality showed a strong dependence on temperature. Although temperature sensitivity was limited to larval stages, the actual deaths occurred at the pupal stage. Survivors, which could be recovered by decreasing the temperature or by reducing the proportion of the Birmingham genome present, often showed multiple developmental anomalies and reduced longevity reminiscent of the effects of cell death from radiation damage. Although the genetic damage occurred in dividing imaginal disc cells, the phenotypic manifestations—death and abnormalities—are not observed until later. The survivors also showed gonadal dysgenic (GD) sterility, a well-known characteristic of P-M hybrid dysgenesis. To explain these findings, we suggest that pupal lethality and GD sterility are both caused by massive chromosome breakage in larval cells, resulting from excision and transposition of genomic *P* elements acting as substrate for the transposase.

MANY studies have been conducted on the genetic effects of *P* transposable elements in Drosophila (reviewed by ENGELS 1983 and BREGLIANO and KIDWELL 1983). Most of this work has been restricted to germline effects, since *P* elements are not normally active in somatic cells. Even the hypermutable *P* element insertion at the *singed* bristle locus, sn^{w}, failed to produce significant somatic mosaicism (ENGELS 1979a), and large scale screens for P-induced somatic recombination (THOMPSON, WOODRUFF and SCHAEFFER 1978; MCELWAIN 1986) indicated only trivial frequencies of such events.

The most conspicuous effect of *P* element activity is known as gonadal dysgenic (GD) sterility (ENGELS and PRESTON 1979; KIDWELL and NOVY 1979; SCHAEFFER, KIDWELL and FAUSTO-STERLING 1979). When males from a strain containing active *P* elements ("P strains") are crossed to females lacking them ("M strains") the resulting progeny of both sexes display temperature-sensitive sterility characterized by small, agametic gonads. This effect is essentially absent at rearing temperatures below 22° and increases with increasing temperature up to 29°. The temperature sensitive period begins in the late embryonic stages and continues through the first and second instars. The detailed mechanism of GD sterility is not known, but has been interpreted as early death of cells caused by transposition and excision of *P* elements. The limitation of *P* element activity to the germline is assumed to be the reason cell death results in sterility rather than death of the organism.

Recent work by LASKI, RIO and RUBIN (1986) reveals that this tissue specificity is controlled at the level of RNA processing. When the third intron of the *P* element's transposase gene was removed *in vitro*, thus eliminating the need for one of the RNA splices, the resulting element caused high levels of mosaicism for *P* element insertion mutations and also for genes inserted within *P* elements. This result indicated that transposase was being produced somatically by the modified element. LASKI, RIO and RUBIN (1986) named their modified *P* element Δ2-3 since the intron bridging exons 2 and 3 had been removed.

In the present study we examine the effects of somatic dysgenesis by taking advantage of a particular insertion of the Δ2-3 element that has the following very useful properties: (1) It is associated with exceptionally high levels of transposase activity as measured by either somatic mosaicism or germline mutability. (2) Recent work (H. ROBERTSON and W. ENGELS, unpublished data) indicates that some *P* elements produce an altered form of the transposase which acts as

a powerful repressor of hybrid dysgenesis. The Δ2-3 element produces no detectable activity of this repressor. (3) The Δ2-3 element is extraordinarily stable, failing to respond to its own transposase. A negligible rate of transposition and excision is essential for experimental manipulation (ROBERTSON *et al.* 1988).

We find that somatic *P* element activity can have devastating consequences to the organism. Analysis of these effects suggests that they can be considered as the somatic equivalent of GD sterility. However, the Δ2-3 element alone is not sufficient to produce these effects. They only occur in the presence of movable *P* elements, elsewhere in the genome.

MATERIALS AND METHODS

Drosophila stocks: All genetic symbols not otherwise explained are in LINDSLEY and GRELL (1968).

sn^{w}; *bw*; *st* (M). The *X* chromosome carries a hypermutable *P* element insertion at the singed bristle locus that is often used as an indicator of *P* element transposase. Previous work has shown that *sn*^{w} mutates at high frequencies to *sn*^{e}, a more extreme form, and to a nearly normal phenotype designated *sn*$^{(+)}$, but only in the presence of autonomous *P* elements (ENGELS 1979a, 1984; SPRADLING and RUBIN 1982). The "M" designation indicates that this strain has no *P* elements other than those at or near *singed*, and has no detectable repressor.

Muller-5, *w*^{a} *B—Birmingham.* This strain is hereafter designated Birmingham, with its *X*, second and third chromosomes designated *Birm1*, *Birm2* and *Birm3*. This stock carries the Muller-5 balancer *X* chromosome and was maintained for several years in the laboratory of M. KEARSEY at the University of Birmingham, England. Despite its classification as an M strain by standard genetic methods, molecular methods later revealed many *P* elements in its genome (BINGHAM, KIDWELL and RUBIN 1982). *In situ* hybridization of *P* element DNA to the polytene chromosomes showed approximately 20 elements on each of the major chromosomes [J. LIM cited by SIMMONS *et al.* (1987), and confirmed in this laboratory]. The presence of *P* elements without the genetic properties that normally accompany them suggests that they are all defective, producing neither transposase nor repressor. O'HARE and RUBIN (1983) describe a class of internally deleted *P* elements that would be expected to be defective in this way. Strains of this kind have been called "M′" or "pseudo-M" strains. Other laboratory stocks with the *M-5* balancer do not have homology to *P* elements as indicated by genomic Southerns or *in situ* hybridization. The *P* elements in the Birmingham stock were presumably derived from wild populations which had been crossed to the balancer stock at some point. Such outcrossing is standard practice in M. KEARSEY's laboratory (personal communication) to maintain the vigor of stocks. There must have been active transposition at first, since the stock now has approximately 20 elements on its Muller-5 chromosome which could not have gotten there by standard meiotic recombination. The original markers, *w*^{a} and *B* remain, and cytological examination of the salivary chromosomes of *Birm1* confirmed that the standard *M-5* rearrangements were still present. The complete *P* elements were probably lost stochastically by segregation, excision, and element-internal deletions which convert complete *P* elements into defective ones (VOELKER *et al.* 1984; DANIELS *et al.* 1985). Stochastic loss of complete elements may have been abetted by greater fertility of those flies having the least transposase. The stock was later inbred for 23 generations by full-sib matings in this laboratory. We believe it was polymorphic prior to this inbreeding, since we find little or no *P* element repressor production in this stock (H. ROBERTSON and W. ENGELS, unpublished data), whereas significant repressor activity was found in a parallel stock maintained in the laboratory of SIMMONS *et al.* (1987).

Stocks were made in which each of the major chromosomes of Birmingham was isolated and the remainder of the genome replaced by M strain chromosomes. We used *in situ* hybridization to a *P* element sequence to confirm that each stock had *P* elements on the Birmingham-derived chromosome and not elsewhere:

Birm1; *bw*; *st.* The *X* chromosome is the *M-5* balancer derived from the Birmingham stock, and the M-derived autosomes are homozygous for eye color markers.

Birm2; *TM6*, *Ubx/Sb.* The M-derived third chromosomes have dominant markers.

CyO/bw^{V1}; *Birm3.* The M-derived second chromosomes have dominant markers.

ry^{506} *P*[*ry*$^{+}$ Δ*2-3*](*99B*). Hereafter designated Δ2-3. This is one of several independent inserts of a *P* element marked with *ry*$^{+}$ and deleted for its third intron. The construction of the element and the finding that it produces P transposase somatically are described by LASKI, RIO and RUBIN (1986). Several Δ2-3 stocks had been maintained in the laboratory of G. RUBIN for approximately 1 yr by backcrossing to *TM3* *ry*^{RK}/*ry*^{506} females and selecting *ry*$^{+}$ sons. When we obtained the stocks we made one of them (designated "3-2" by LASKI, RIO and RUBIN) homozygous for the Δ2-3 element by inbreeding and selecting by progeny testing and kin selection for the *ry*$^{+}$ phenotype and somatic transposase activity. *In situ* hybridization showed the element to be at cytological position 99B7-10 on the right arm of chromosome *3*; no other points of *P* element homology were seen. After maintaining the homozygous Δ2-3 stock for several generations and using it in experiments, we noticed a conspicuous lack of any excision of the Δ2-3 element at 99B or appearance of new copies elsewhere. This stability was all the more striking because of the apparent high levels of transposase indicated by germline and somatic mobility of nonautonomous elements, and was the first indication that this *P* element was abnormally stable even in the presence of transposase. This stability is implied by some of the results described below, and more fully documented by ROBERTSON *et al.* (1988).

C(1)DX, *y f*; *ry*^{506} *P*[*ry*$^{+}$ Δ*2-3*](*99B*). Similar to above except that females carry an attached *X* chromosome and a *Y* chromosome. This stock is sometimes abbreviated *C(1)DX*, *y f*; Δ*2-3*.

ry^{506}. This stock is identical (or nearly so) to the stock used as the recipient for injection of *P*[*ry*] elements by KARESS and RUBIN (1984) and by LASKI, RIO and RUBIN (1986). We therefore assume that it is essentially isogenic with the Δ2-3 stock (above) except for lacking the *P* element on chromosome *3*. There are no *P* elements in the *ry*^{506} stock.

C(1)DX, *y f*; *ry*^{506}. Similar to above except that females carry an attached *X* chromosome and a *Y* chromosome.

Cy/bw^{V1}; *D/Sb.* A normal M strain (no *P* elements) carrying dominant markers on chromosomes *2* and *3*.

Pupal lethality measurements: Pupal lethality was measured by the frequency of noneclosion, unless otherwise noted. For example, matings of Δ2-3 ♀ × Birmingham ♂ and control crosses consisting of the same males mated to *ry*^{506} females were set up at room temperature. After 1–3 days to allow time for mating they were moved to fresh medium

and incubated at the temperature appropriate to the experiment, usually 28°. In most cases they were transferred again a day or two later after acclimatization to the new temperature. The adults were removed after 12–48 hr (longer when at lower temperatures). When all progeny had eclosed from the control vials, which usually occurred after 10–11 days at 28°, the experimental vials were kept for an additional 48 hr or more, at which time noneclosed pupae in the experimental vials were assumed to be dead. We then scored all pupa cases on the sides of the vials as either eclosed (empty) or dead.

***In situ* hybridization:** Slides were prepared, hybridized and labeled with biotinylated DNA probes as described (ENGELS *et al.* 1986). In most cases, the probe was the complete *P* element sequence containing flanking DNA from the 17C region (O'HARE and RUBIN 1983). Otherwise, we used a subclone provided by K. O'HARE (personal communication) containing nearly all of the complete *P* element sequence (from the leftmost *Hin*dIII site to the rightmost *Ava*II site on O'HARE and RUBIN's (1983) map).

RESULTS

Initial observations: Qualitative observations in pilot experiments showed no trace of GD sterility or somatic ill effects when Δ2-3 flies were crossed to M strains in either direction, even at 28°. Moreover, the Δ2-3 stock itself showed no GD sterility or other abnormalities when maintained at 28°. These findings seemed inconsistent with the high levels of transposase activity associated with Δ2-3. As an indication of its highly active transposase, a single copy of the Δ2-3 element has been observed to cause close to 100% of sn^w males to display somatic bristle mosaicism [LASKI, RIO and RUBIN (1986), confirmed in this laboratory]. Examination of the next generation showed that the germline of these sn^w;Δ2-3/+ males was also highly mutable: the combined rate to the two kinds of secondary mutations (sn^e and sn^+) approached 100% as the males aged (ROBERTSON *et al.* 1988). This is a much higher level of mutability than had been previously observed for sn^w even when many *P* elements were available to provide transposase (ENGELS 1979a, 1983; RAYMOND and SIMMONS 1981; SIMMONS *et al.* 1985).

One way to reconcile these observations is to suppose that GD sterility and other dysgenic effects require not only transposase, but also a set of "target" *P* elements to serve as substrate. We tested this idea by crossing Δ2-3 to the Birmingham strain in both directions. Birmingham was expected to be a good source of target elements, since its chromosomes contain approximately 60 sites of *in situ* hybridization to *P* element probes, yet it behaves as an M strain (BINGHAM, KIDWELL and RUBIN 1982).

No progeny from these crosses survived beyond the pupal stage, regardless of rearing temperature (19–28°) or direction of the cross. Some survivors were seen when subsets of the Birmingham genome were used, but even then only at reduced temperatures.

These survivors exhibited multiple anomalies. The most common were GD sterility; distorted head and eye shapes; patches of missing abdominal tissue; missing bristles, hairs and ocelli; eyes with rough surfaces and reduced size, and tumorous growths of amorphous tissue. There were also cases of mirror-image duplicated segments. These traits were similar to abnormalities previously reported by several authors in adults whose larvae had been subjected to high doses of radiation (WADDINGTON 1942; VILLEE 1946; POSTLETHWAIT and SCHNEIDERMAN 1973; POSTLETHWAIT 1975; HAYNIE and BRYANT 1977). They attributed these anomalies to cell death and compensatory growth of surviving cells in imaginal discs and histoblast nests. There are, however, some possible differences between the Δ2-3/Birmingham syndrome and the irradiated flies. In our material abdominal defects were highly conspicuous while mirror image duplications were relatively rare. Published descriptions of irradiated flies cited above, on the other hand, suggest the reverse.

Individual chromosomes of Birmingham: To map the responsible factor(s) in the Birmingham genome, we crossed Birmingham females to Cy/bw^{V1}; *D/Sb* males. Each of 92 sons in which Birmingham autosomes were opposite dominant markers, was crossed individually to either Δ2-3 or ry^{506} females. The progeny were reared at 19°, 21° or 28° and all progeny were scored for sex and the dominant markers. Additional data for the autosomes were obtained by collecting 162 sons from the cross of Cy/bw^{V1}; *D/Sb* females to Birmingham males and mating them to Δ2-3 or ry^{506} as above and scoring the progeny in the same way. The frequencies of recovery of the Birmingham chromosomes among the progeny of the Δ2-3 females as compared to progeny of ry^{506} females provided an indication of which part(s) of the Birmingham genome were required for the lethal interaction.

The control matings (ry^{506} mothers) indicated that there were no major viability differences associated with sex (1486 males: 1432 females) or any of the dominant markers (*Cy*:+ = 607:602; bw^{V1}:+ = 827:821; *D*:+ = 604:542; *Sb*:+ = 830:831). We therefore assumed that no significant viability effects came from the dominant markers, and pooled the data over these categories to yield the totals in Tables 1 and 2.

We see that each of the major Birmingham chromosomes was lethal at 28° in the presence of Δ2-3 and severely deleterious at lower temperatures. Chromosome *3* had the most pronounced effect, followed by the *X* chromosome, with chromosome *2* being the least deleterious.

Note from Tables 1 and 2 that combinations of Birmingham chromosomes appear to be more deleterious than the corresponding individual chromo-

TABLE 1

Recovery of Birmingham *X* and autosomes in the presence of Δ2-3

Group	$\frac{mm\Delta}{YMM}$[a]	$\frac{mm\Delta}{BMM}$	$\frac{mm\Delta}{YBM}$	$\frac{mm\Delta}{YMB}$	$\frac{mm\Delta}{BBM}$	$\frac{mm\Delta}{BMB}$	$\frac{mm\Delta}{YBB}$	$\frac{mm\Delta}{BBB}$	Total progeny	No. of crosses
19° ry	20	25	24	25	30	29	30	27	210	4
Δ2-3	213 (*202.0*)[b]	58 (*64.1*)	112 (*129.1*)	2 (*1.8*)	48 (*41.0*)	0 (*0.6*)	7 (*0.0*)	0 (*0.0*)	440	29
21° ry	47	57	48	46	45	59	59	52	413	4
Δ2-3	397 (*409.2*)	26 (*25.9*)	202 (*191.0*)	2 (*0.5*)	12 (*12.1*)	0 (*0.0*)	0 (*0.0*)	0 (*0.0*)	639	26
28° ry	3	8	0	2	3	11	4	2	33	3
Δ2-3	9 (*9.0*)	0 (*0.0*)	0 (*0.0*)	0 (*0.0*)	0 (*0.0*)	0 (*0.0*)	0 (*0.0*)	0 (*0.0*)	9	6

The observed numbers of each Birmingham chromosome combination in the presence of Δ2-3 or ry^{506} are shown for each indicated temperature. The father's genotype was: *Birm1/Y*; *Birm2/M*; *Birm3/M*, where *M* indicates a dominantly marked chromosome with no *P* elements.

[a] Maternal genotype for three major chromosomes is shown on top and paternal genotype on the bottom: the *X*, second and third chromosomes are designated "*B*" for Birmingham stock, "*M*" for dominantly marked M strain, and "*m*" or "Δ" for the Δ2-3 strain (or the ry^{506} strain in the case of the controls).

[b] Parentheses indicate expected number based on independent action of the Birmingham chromosomes. See APPENDIX and Table 3.

TABLE 2

Recovery of Birmingham autosomes in the presence of Δ2-3

Group	$\frac{mm\Delta}{(M \text{ or } Y)MM}$[a]	$\frac{mm\Delta}{(M \text{ or } Y)BM}$	$\frac{mm\Delta}{(M \text{ or} Y)MB}$	$\frac{mm\Delta}{(M \text{ or } Y)BB}$	Total progeny	No. of crosses
19° ry	118	139	166	139	562	9
Δ2-3	845 (*843.1*)[b]	542 (*538.7*)	1 (*7.4*)	6 (*4.7*)	1394	28
21° ry	231	237	262	258	988	8
Δ2-3	1106 (*1094.9*)	501 (*511.0*)	1 (*1.5*)	0 (*0.7*)	1608	60
28° ry	164	155	161	171	651	12
Δ2-3	38 (*38.0*)	0 (*0.0*)	0 (*0.0*)	0 (*0.0*)	38	45

The observed numbers of each Birmingham autosome combination in the presence of Δ2-3 or ry^{506} at the indicated temperature. The father's genotype was: *M/Y*; *Birm2/M*; *Birm3/M*.

[a,b] Same as Table 1.

somes. The simplest explanation is that each Birmingham chromosome carries one or more factors acting independently of each other (but synergistically with Δ2-3) to cause lethality. Independence implies multiplicativity, such that the probability of surviving any combination of Birmingham chromosomes is the product of the individual survival probabilities. Based on this model, we obtained maximum likelihood estimates of the individual chromosome effects (see APPENDIX) and used them to compute the expected number of progeny of each class in the experimental matings (Δ2-3 mothers).

The maximum likelihood estimates are shown in Table 3. From the top half of the table we see that in the absence of Δ2-3 each Birmingham chromosome has a fitness of approximately unity at all temperatures. (The only exception is the *X* chromosome at 28° where the sample size was extremely small.) However, when Δ2-3 was present (lower half of Table 3) each chromosome was severely deleterious, especially at elevated temperatures. These values also reflect the much stronger effect from chromosome *3* as compared to the *X* and second chromosomes.

The expected numbers based on this model are shown in parentheses in Tables 1 and 2. The remarkably good fit to the observed numbers suggests that the responsible factors on the Birmingham chromosomes act approximately independently of each other, with no detectable synergism.

TABLE 3

Survival of Birmingham chromosomes relative to M chromosomes

Mothers	Temp.	Chromosome *X*	Chromosome *2*	Chromosome *3*
ry^{506}	19°	1.121[a]	1.016	1.169
	21°	1.065	0.996	1.107
	28°	2.667	0.960	1.054
Δ2-3	19°	0.317	0.639	0.009
	21°	0.063	0.467	0.001
	28°	0.000	0.000	0.000

Maximum likelihood estimates of survival probabilities for each Birmingham chromosome with or without Δ2-3 present. Calculations, as described in the APPENDIX, were based on the data in Tables 1 and 2.

[a] Values greater than 1 indicate that the Birmingham chromosome was recovered more frequently than the M chromosome.

Effects of the Birmingham *X* chromosome when

hemizygous: In the previous experiment, all Birmingham chromosomes were in the heterozygous state when tested with Δ2-3. The resulting lethality can therefore be considered dominant. To examine the hemizygous state, we crossed *Birm1*; *bw*; *st* females to Δ2-3 males (or ry^{506} males for controls) at 19° and scored the sex of the progeny. We observed a preponderance of daughters (465 ♀:34 ♂) in the experimental group but not in the controls (239 ♀:211 ♂) indicating that the Birmingham *X* chromosome is more effective in causing pupal lethality when hemizygous than heterozygous. Our interpretation is that some of the lethal effects of the Birmingham/Δ2-3 combination can be recessive in somatic cells. Similar explanations have been applied to the observation that males are more sensitive than females to pupal lethality resulting from larval exposure to X-rays (OSTER and CICAK 1958).

Lack of autonomous *P* elements in the Birmingham strain: Our interpretation of the above experiments is based on the assumption that Δ2-3 is the sole source of *P* element transposase in the Δ2-3/Birmingham hybrids. As mentioned earlier, Birmingham was classified genetically as an M strain, meaning that it had no detectable repressor or transposase. However, the standard tests for classification in the P-M dysgenesis system might not detect small amounts of transposase (JONGEWARD, SIMMONS and HEATH 1987) or repressor (H. ROBERTSON and W. ENGELS, unpublished data). Therefore we retested the stock using more sensitive techniques. The repressor tests to be described elsewhere (H. ROBERTSON and W. ENGELS, unpublished data) indicated no significant repressor on the Birmingham autosomes. The test for transposase was performed as follows:

Females from the sn^w; *bw*; *st* (M) strain were mated to Birmingham males. Each of 197 sons was individually testcrossed to compound-*X* females to screen for mutations to sn^e or sn^+. Only one of 17,014 sons had a phenotype different from sn^w. This male resembled sn^e, but failed to reproduce. The mutation was therefore unconfirmed. Similarly, 196 daughters from the original cross, whose genotype was *M-5*,w^a *B*/sn^w, were mated to males carrying sn^3, a recessive allele causing extreme *singed* bristles. B^+ progeny of both sexes were scored for *singed* mutations. Among 21,949 B^+ progeny, there were six that appeared to be sn^+. However, cytological inspection showed that each of the six carried part of the *M-5* rearrangement, indicating that they were actually the result of double crossovers. We conclude that there is no detectable transposase produced by the Birmingham chromosomes. Similar experiments by SIMMONS *et al.* (1987) are in good agreement.

Developmental stage of lethality: To determine the developmental stage at which the Δ2-3/Birmingham interaction causes death, we crossed males from the *Birm2*; *TM6/Sb* stock to either Δ2-3 or ry^{506} females. Eggs were collected on small petri plates containing medium and allowed to develop at 16°, 19°, 22°, 25° and 28°. The *Birm2*; *TM6/Sb* stock was used in this experiment rather than the Birmingham stock itself so that the mortality would be less than 100% at the lower temperatures, thus allowing greater sensitivity to detect subtle differences. In addition, it was important to avoid the Birmingham *X* chromosome to ensure that all progeny have the same number of Birmingham chromosomes (and that none be hemizygous for *Birm1* if the reciprocal cross were used). Plates were examined daily to determine the number of individuals surviving to each developmental stage. Embryonic mortality rates were measured as the percentage of eggs that failed to hatch. Larval mortality is the percentage of larvae that did not form pupae, and pupal mortality is the percentage of these pupae that failed to eclose.

The results are in Figure 1, and the sample sizes are in the legend. It is clear that the experimental group differs substantially from the controls only in the pupal stage. Furthermore, this difference is much more severe at higher temperatures. Although it is still possible that some lethal effects occur at earlier stages, we conclude that the bulk of the lethality occurs in pupae.

Adult longevity: We noticed in some of the previous experiments that the surviving adults with Δ2-3 and Birmingham chromosomes did not appear to live as long as the controls. To examine this effect, we mated *Birm2*; *TM6/Sb* males to either Δ2-3 or ry^{506} females at 16°. From each cross, we collected 100 females immediately after eclosion and placed them ten per vial at 28°. At 3-day intervals they were transferred to fresh vials and the number of survivors was recorded. Only females were used in this experiment so that the background genotype of the control and experimental groups would be identical except for the Δ2-3 element.

The results in Figure 2 show that the mortality rate is much greater in the experimental group as compared to the controls. The death at the adult stage, however, may be the result of P activity at a much earlier time.

Temperature effect: The data discussed above (Tables 1–3) indicate a strong dependence of lethality on temperature. We now examine this effect in more detail by looking at one Birmingham chromosome at a time. Matings of Δ2-3 females to males from each of the three stocks in which one of the major Birmingham chromosomes had been isolated, were placed at 16°, 19°, 21°, 25° and 28°. For controls, ry^{506} females were mated to Birmingham males at the same set of temperatures. In addition, to examine the

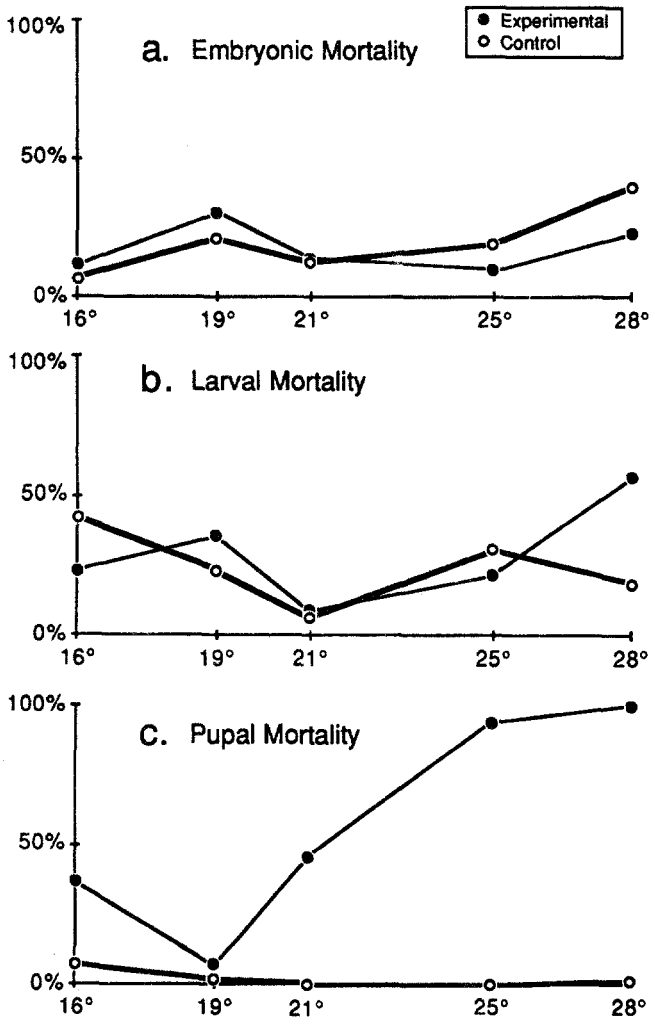

FIGURE 1.—Mortality rates at three stages of development as a function of temperature. The solid circles (●) are from the cross Δ2-3 ♀ × *Birm2*; *TM6/Sb* ♂, and the open circles (○) are ry^{506} ♀ × *Birm2*; *TM6/Sb* ♂. Average sample sizes for embryo mortality are 172 embryos per data point of the Δ2-3 group, and 154 for the controls. Larval sample sizes are 127 larvae per data point in the Δ2-3 group and 120 in the controls. Pupal sample sizes were 107 pupae per data point in the Δ2-3 group and 91 in the controls.

effect of temperature on the hemizygous Birmingham *X* chromosome, we crossed *C(1)DX, y f*; *Δ2-3* females to *Birm1*; *bw*; *st* males at the same temperatures with *C(1)DX, y f*; ry^{506} females used as controls. Pupal lethality was measured as described above for the two autosomal stocks and their controls. For the crosses involving the Birmingham *X* chromosome, where "target" *P* elements are expected only in the daughters (or only in the sons in the case of the attached-*X* crosses), lethality is measured as the sex ratio among the eclosed adults. This quantity should be comparable to the autosomal measurements provided most of the lethality is at the pupal stage and there are no major sex differences in viability.

As expected, the results in Figure 3 show that pupal lethality is highly temperature sensitive, with each of the three chromosomes showing sharply decreasing

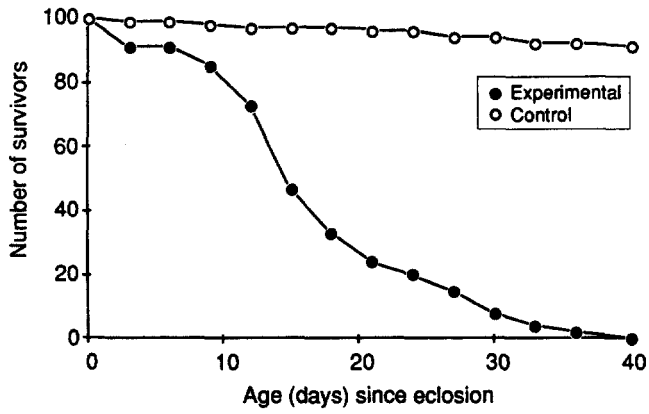

FIGURE 2.—Adult survival. Survival at 28° of 100 adult females from the cross of *Birm2*; *TM6/Sb* males to Δ2-3 females (●) or ry^{506} females (○). Both sets were raised at 16° until eclosion.

survival with increasing temperature. They also show that the Birmingham chromosomes differ in their effects. Chromosome *3* is almost completely lethal at 19° but had substantial numbers of survivors at 16°. The effects of chromosome *2* were much milder, with full lethality only at temperatures exceeding 25°. The *X* chromosome was intermediate when heterozygous but highly lethal when hemizygous, especially at temperatures over 19°. The controls indicated no pupal lethality at any temperature in the absence of Δ2-3, even with all three major chromosomes of Birmingham present.

GD sterility: As mentioned above, we found no evidence of GD sterility within the Birmingham or Δ2-3 stocks or in their progeny when crossed to ordinary M strains. To determine whether the Δ2-3/Birmingham interaction could cause GD sterility, surviving females from the previous experiment were kept for five days to allow full gonadal development, then dissected and scored for GD sterility. Figure 4 shows that GD sterility follows the same pattern as pupal lethality. It became more severe with increasing temperature, and the Birmingham *X* chromosome had a greater effect than chromosome *2*. (No data are available for chromosome *3* since the there were too few survivors.)

Note that GD sterility can be produced by the Δ2-3/Birmingham combination at much lower temperatures than it can with standard P strain × M strain crosses such as those studied by KIDWELL and NOVY (1979) and ENGELS and PRESTON (1979). In the earlier studies there was no indication of GD sterility at temperatures below 22°, whereas the present results indicate that Δ2-3 produces the effect at considerably lower temperatures when Birmingham chromosomes are present.

Period of temperature sensitivity: The developmental timing of temperature sensitivity was determined by temperature shift-up and shift-down experiments. Small mass matings of Δ2-3 females to *Birm2*;

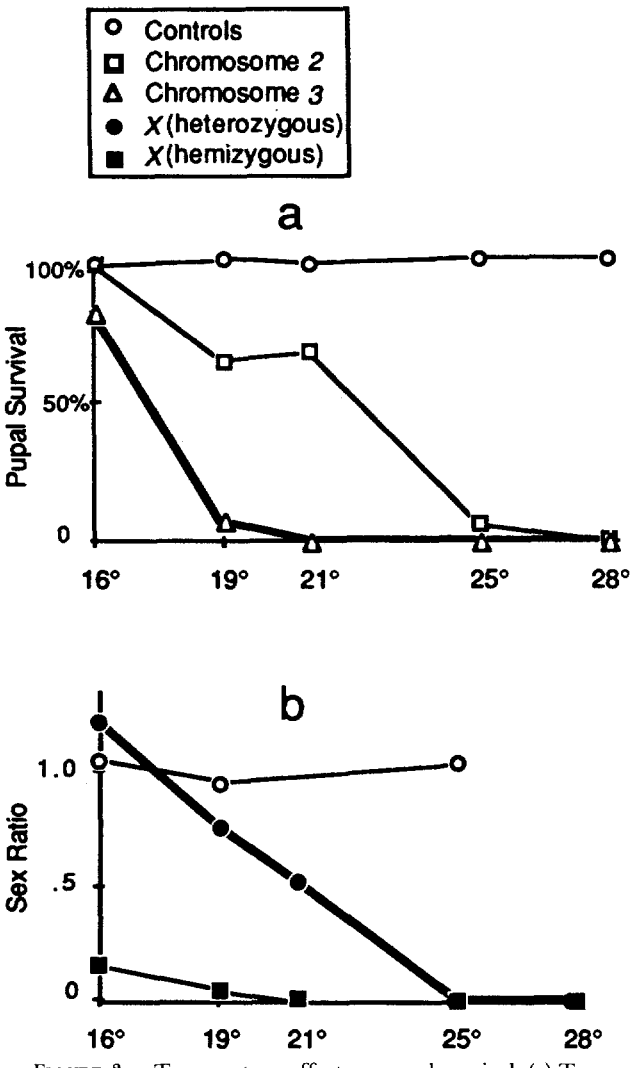

FIGURE 3.—Temperature effect on pupal survival. (a) Temperature effect of Birmingham autosomes on survival. The proportion of pupae that eclose is plotted against rearing temperature. The average sample size per data point was 184 pupae. (○) Controls from the mating of ry^{506} ♀ × Birmingham ♂; (□) Birmingham chromosome 2 only. The cross was Δ2-3 ♀ × *Birm2*; *TM6/Sb* ♂; (△) Birmingham chromosome *3* only. The cross was Δ2-3 ♀ × *CyO/* bw^{V1}; *Birm3* ♂. Note that the 19° test of chromosome *2* seems to indicate survival rate that is somewhat lower than expected from the overall shape of the curve. Other experiments, some of which are described elsewhere in this article, indicate that survival of this genotype/temperature combination is actually in the 85–90% range, which would fit much better. A likely explanation for this discrepancy is instability of the incubator temperature. (b) Temperature effect of Birmingham *X* chromosomes. Survival was measured by sex ratio with the numerator being whichever sex has *Birm1*. (○) Control ratios of males/females from the cross *C(1)DX, y f;* ry^{506} ♀ × *Birm1* ♂. The average sample size was 193 adults per point. (●) Heterozygous effects measured by the ratio of females/males from the cross Δ2-3 ♀ × *Birm1* ♂. The average sample size was 89 adults per data point. (■) Hemizygous effects measured by the ratio males/females from the cross *C(1)DX, y f;* Δ*2-3* ♀ × *Birm1* ♂. The average sample size was 242 adults per data point.

TM6/Sb males were set up for pre-mating as described in MATERIALS AND METHODS. We chose the *Birm2*; *TM6/Sb* stock instead of the Birmingham stock itself

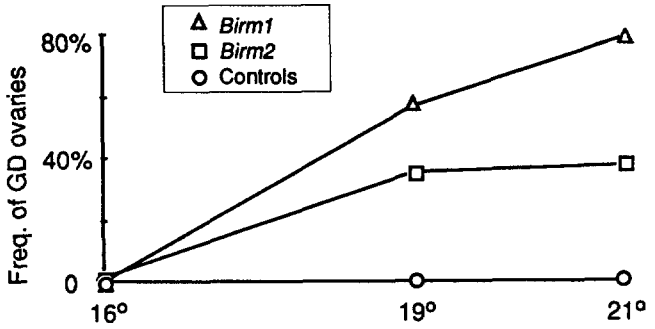

FIGURE 4.—GD sterility. The ordinate measures the proportion of ovaries among the dissected females showing the characteristic rudimentary phenotype of GD sterility. Each point represents an average sample size of 170 ovaries (85 dissections). (△) Birmingham *X* chromosome only; (□) Birmingham chromosome *2* only; (○) Controls having complete Birmingham genome, but mated to ry^{506} instead of Δ2-3. Survival was insufficient to provide data for temperatures greater than 21° or for chromosome *3* at any temperature.

for the same reasons as previously. Controls were ry^{506} females mated to *Birm2*; *TM6/Sb* males.

For the shift-down experiment, vials containing experimental and control matings were placed at 28° where eggs were deposited for 2 hr before the adults were removed. During the same 2 hr, another set of control matings was left to oviposit on small petri plates containing medium. These plates were examined at each time point to determine the proportion of eggs that had hatched, thus allowing us to calibrate the developmental rate and to confirm that the eggs were synchronized.

Four vials from the experimental group and two from the control group were moved from 28° to 19° at each time point. They were then left to complete development at 19° until pupal mortality was measured as described previously.

The shift-up experiment was similar except that (1) matings were initially set up at 19° and moved at various time points to 28°. (2) Eggs were deposited for 4 hr instead of 2 hr. (3) Pupariation was used as the developmental landmark rather than egg hatching, since pilot experiments suggested the end of the temperature sensitive period would be at approximately that time.

Results of the shift-down experiment are in Figure 5a and the sample sizes are in the legend. The results show that there is an initial period of insensitivity to high temperature. Sensitivity begins a few hours after the eggs hatch. Full pupal lethality results when the first 100 hr of development are at 28°. The shift-up experiment (Figure 5b) shows that sensitivity to temperature continues throughout larval development, tapering off about the time of puparium formation.

In this experiment we measured mortality only at the pupal stage, yet sensitivity to temperature occurred much earlier. We conclude that irreversible lethal events occur during larval development which

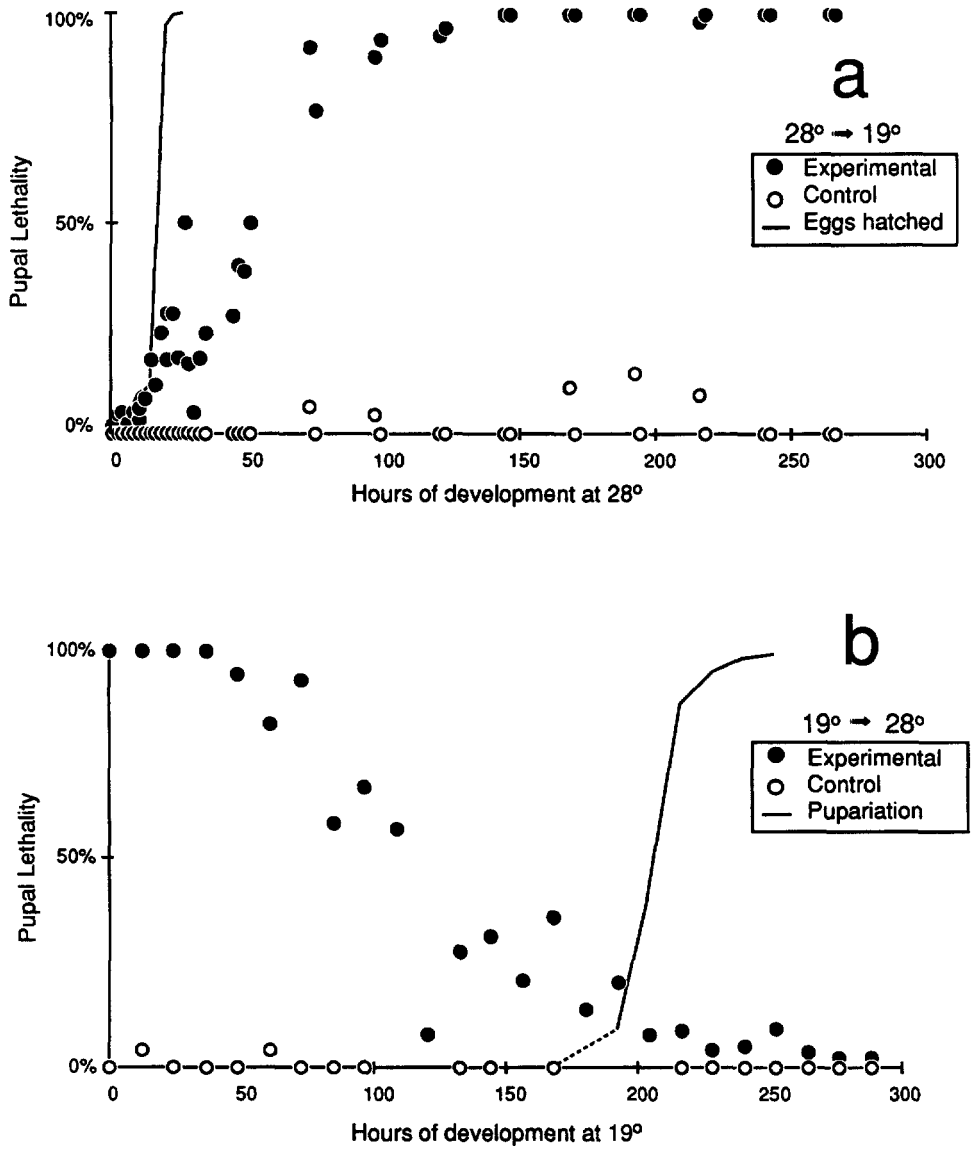

FIGURE 5.—Frequency of pupal lethality after temperature shifts. Solid circles (●) represent the average of pupal lethality measurements for all experimental vials (Δ2-3 mothers) shifted at the indicated time. Open circles (○) are the same for the control vials (ry^{506} mothers). (a) Shift-down experiment. Each point represents an average of 31 pupae scored for the experimental group, and 11 pupae for the controls. The solid line is the cumulative proportion of eggs that had hatched by the indicated time at 28° based on a sample of 198 eggs. (b) Shift-up experiment. Each point represents an average of 99 pupae for the experimental group and 11 for the controls. The solid line is the cumulative proportion of puparia formed by the indicated time based on a sample of 145 pupae from the control group.

do not affect the viability of the larvae, but nevertheless cause death at the pupal stage.

Reciprocal cross effects: Traits associated with hybrid dysgenesis normally show a reciprocal cross effect (KIDWELL, KIDWELL and SVED 1977) resulting from a regulation mechanism known as cytotype (ENGELS 1979b). This regulation is now thought to be based upon a *P* element-encoded repressor product. The present situation is more ambiguous than the usual case of P strains crossed to M strains. Both Δ2-3 and Birmingham strains are of the M cytotype (*i.e.*, lacking in P repressor), and both contribute *P* elements to the affected offspring, although only the Δ2-3 element is expected to produce transposase.

As mentioned earlier, the cross of Δ2-3 by Birmingham produces total pupal lethality in both directions. However, it is still possible that there is a slight quantitative reciprocal cross difference that could not be detected in the preliminary experiments due to the completeness of the lethality. To test this possibility we performed reciprocal crosses in a way designed to yield less than 100% pupal lethality, thus increasing the sensitivity to detect slight reciprocal differences.

Δ2-3 was crossed to *Birm2*; *TM6/Sb* in both directions and the progeny were raised at 25°. The use of only the *Birm2* chromosome and the less extreme rearing temperature are expected to produce the desired intermediate level of lethality, which was measured as described previously.

The results are shown in Figure 6. Both crosses produced high levels of pupal lethality: 95% in the case of Δ2-3 mothers (2093/2195) and 98% in the case of the *Birm2*; *TM6/Sb* mothers (3639/3727). It is clear that the two distributions overlap extensively. However, there is a tendency for more lethality in the case of *Birm2*; *TM6/Sb* mothers that is significant at

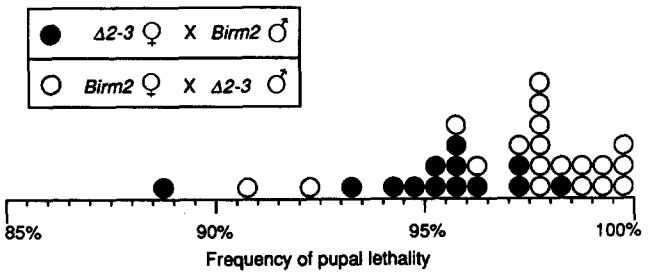

FIGURE 6.—Reciprocal cross tests. Each solid circle (●) represents one of the replicate crosses of Δ2-3 ♀ × *Birm2*; *TM6/Sb* ♂. The frequency of pupal lethality was computed from an average of 169 pupae per replicate. The open circles (○) are replicate vials of the reciprocal cross, averaging 196 pupae scored per data point.

the 0.01 level by the rank sum test, with each replicate vial treated as an independent trial. This reciprocal difference, though real, is so small that it might have a trivial explanation. For example, the males in the two groups differ in their *X* chromosomes. Although they are expected to have the same complement of *P* elements, their genetic backgrounds will differ, possibly causing different susceptibility to the pupal lethality.

Identity of the Δ2-3 component: The following two experiments demonstrated that the relevant genetic component in the Δ2-3 stock is the Δ2-3 *P* element itself. First, a mapping experiment showed that the lethal effect is not readily separable from the ry^+ marker on the Δ2-3 transposon. We crossed heterozygous females of the genotype ry^{506}/ry^{506} *P*[ry^+ Δ2-3] (*99B*) to *Birm2*; ry^{506} males at 28° and scored *rosy* phenotypes of the survivors. The controls, which were similar except that ry^{506} males without any Birmingham chromosomes were used, produced 80 *rosy* and 288 ry^+ progeny. The deviation from 1:1 recovery reflects the reduced survivorship of the *rosy* phenotype at elevated temperatures (LINDSLEY and GRELL 1968). In the experimental group there were 168 *rosy* and no ry^+ progeny. Using the control data we estimate that there were approximately 168(288/80) = 605 ry^+ embryos that did not survive. We conclude that the pupal lethality function maps extremely close to the ry^+ marker on the transposon.

The second experiment tests whether other integrations of the Δ2-3 transposon also cause pupal lethality. F. LASKI, D. RIO and G. RUBIN provided us with two other stocks with integrated *P*[ry^+ Δ2-3] transposons derived from independent microinjections. These stocks were maintained in this laboratory by inbreeding and selection for ry^+. They were characterized by two sets of *in situ* hybridization tests performed approximately 1 yr apart. One of the stocks (designated "8") was apparently stable, having only a single site at 68C seen in each of three larvae in the first set of tests and nine larvae in the second. The other ("10-1") was clearly heterogeneous with sites observed at 16F, 79C, 84E, 86C and 94D in various larvae. No larva had more than two sites, and several had none.

TABLE 4

Pupal lethality using other Δ2-3 lines

Line	Temperature	Vials scored	Pupae scored	Pupal mortality (%)
Δ2-3 (8)	16°	16	1392	95.83
Δ2-3 (8)	19°	25	3304	99.97
Δ2-3 (8)	22°	39	5138	99.96
Δ2-3 (8)	25°	88	8054	100.00
Δ2-3 (10-1)	19°	15	2364	48.65
Δ2-3 (10-1)	22°	15	2411	72.87
Δ2-3 (10-1)	28°	10	575	75.48

Males of the indicated Δ2-3 strains were crossed to *Birm2*;*TM6/Sb* females at the given temperature. Measurements of pupal lethality were as described above.

Males from each stock were crossed to *Birm2*; *TM6/Sb* females at each of several temperatures and the vials scored for pupal lethality in the usual way. The results (Table 4) indicate that stock 8 had consistently high levels of pupal lethality. Some pupal lethality was also seen for stock 10-1, but not as consistently, presumably because of the polymorphism for the Δ2-3 element. Since two other integrations of Δ2-3 cause pupal lethality in conjunction with Birmingham chromosomes, we conclude that this lethality is caused by the element *per se*, and is not dependent on the specific insertion at chromosomal position 99B.

Tests of strains other than Birmingham: In the P-M system of hybrid dysgenesis, strains are normally classified as P, M, M′ or Q (KIDWELL, KIDWELL and SVED 1977, KIDWELL and NOVY 1979, KIDWELL 1981; BINGHAM, KIDWELL and RUBIN 1982). These classifications depend on the appearance of dysgenic traits after crosses to standard reference strains, and also on the presence or absence of *P* elements in the genome as detected by molecular methods. Q strains are those that produce little or no GD sterility when crossed to standard P and M strains in either direction; the three other strain types were defined above. This classification is now thought to be an oversimplification, since each of the categories is heterogeneous (JONGEWARD, SIMMONS and HEATH 1987; DANIELS *et al.* 1987). The M′ classification, which includes Birmingham, is especially ambiguous since some M′ strains produce detectable levels of transposase (JONGEWARD, SIMMONS and HEATH 1987) whereas Birmingham does not (SIMMONS *et al.* 1987, and above). Moreover, they are also variable in production of repressor (H. ROBERTSON and W. ENGELS, unpublished data).

With these caveats in mind, we set out to test whether strains in any of the four major categories could substitute for Birmingham in causing lethality when combined with Δ2-3 as a source of transposase

in somatic cells. We selected 51 M strains, so classified by explicit tests, or by inference from their laboratory origin; one strain each of classes P and Q; and four M′ strains other than Birmingham. The P, Q and M′ strains were classified using GD sterility as described by ENGELS and PRESTON (1980) and tested for the presence of *P* elements using *in situ* hybridization. Males from each strain were mated to Δ2-3 females in small mass matings at 25° or 28°. Inspection of the vials after 16 days showed none of the crosses yielded pupal lethality at significant frequencies comparable to Birmingham. If we assume that the four M′ strains carry repressor-making *P* elements as suggested by preliminary tests (H. ROBERTSON and W. ENGELS, unpublished data) then the results are consistent with the notion that pupal lethality requires, in addition to a source of somatic transposase, nonautonomous *P* elements and the absence of repressor.

DISCUSSION

Genetic components: Perhaps the most significant aspect of the effects we describe is that they are strictly dependent on the interaction of at least two genetic components. Neither the Δ2-3 element nor the Birmingham chromosomes causes any detectable pupal lethality, but the combination is deadly.

The causative factor in the Δ2-3 strain is almost certainly the Δ2-3 element itself. This conclusion comes from the following lines of reasoning: First, we see no pupal lethality when the ry^{506} strain is substituted for Δ2-3. These two strains are thought to be essentially isogenic except for the Δ2-3 element. Second, the pupal lethality is very tightly linked to the ry^{+} marker on the Δ2-3 element. Third, at least two other inserts of Δ2-3 elements in different chromosomal locations have been observed to produce pupal lethality, thus ruling out the possibility that the effect is caused by an insertional mutation of a genomic function.

There is also evidence that the responsible genetic factors in the Birmingham strain are the nonautonomous *P* elements. Here, however, the evidence is somewhat more ambiguous. First, the pupal lethality factors map to all the Birmingham major chromosomes, as do the *P* elements seen by *in situ* hybridization. We do not see any pupal lethal effect from M strain chromosomes, which lack *P* elements. One counterargument to this line of reasoning is that if the Birmingham strain arose by outcrossing to natural populations, as postulated above, then it might differ from M strains by unknown factors in addition to *P* elements. Such differences, however, are likely only on the autosomes and *Y* chromosome. This is because the *X* chromosome of Birmingham is the multiply inverted and dominantly marked balancer known as *M-5*. We find that this chromosome still retains the multiple rearrangements and genetic markers that are standard for *M-5*. Therefore, it is unlikely that it has acquired any part of a wild-derived chromosome by standard meiotic recombination. Its *P* elements presumably got there by transposition. Since this *X* chromosome causes pupal lethality whereas standard laboratory *M-5* chromosomes (lacking *P* elements) do not, we can conclude that the causative factors are transposable elements acquired by the *Birm1* chromosome during the same period of time that it acquired *P* elements.

Another potential counterargument to our claim that nonautonomous *P* elements are responsible for pupal lethality is the weakness of the correlation between the severity of the deleterious effect and the number of *P* elements seen as *in situ* hybridization sites. The Birmingham chromosome *3*, for example, causes much more lethality than chromosome *2*, yet it has approximately the same number of sites. J. LIM (cited by SIMMONS *et al.* 1987) reports 17 *P* elements on chromosome *2* and 22 on chromosome *3*. We have observed approximately the same numbers in our strain. The relatively slight difference in number of sites does not seem adequate to account for the large difference we observe in pupal lethality. We believe, however, that this lack of correlation can be explained by hypothesizing that there is variability among *P* elements for their efficacy in causing pupal lethality. Such variability might arise from internal sequence differences between elements and/or from their varying chromosomal positions. We already know, for example, that *P* elements vary widely in their ability to undergo transposition and excision, even when the transposase source is held constant. The Δ2-3 element itself is the best example of a *P* element that appears to have very little ability to transpose or excise in the presence of transposase. In addition, we have a series of other marked *P* element inserts that display a continuum of mobility levels (ROBERTSON *et al.* 1988). One possibility, currently under investigation, is that those elements that are more active in transposition and excision are also the ones that contribute most to the pupal lethality. According to this model, we would expect the Birmingham chromosome *3* to have more of the highly active elements than chromosome *2*. Other reasons for this lack of correlation, such as the possibility of multiple *P* elements at a single *in situ* hybridization site, or *P* elements that do not show up by *in situ* hybridization, are also under investigation.

Finally, we must explain why only Birmingham among several independent M′ strains tested produces the pupal lethal interaction. One explanation, as suggested above, is that some *P* element repressor is produced by the other M′ strains, but not by Birmingham. This repressor is not sufficiently strong to be easily detected in ordinary P × M crosses, but

appears to be very effective against Δ2-3 (H. ROBERTSON and W. ENGELS, unpublished data).

GD sterility: We find that GD sterility follows some of the same rules as pupal lethality. That is, neither the Δ2-3 element nor the Birmingham genome is sufficient by itself to cause either effect, but in combination both traits appear. SIMMONS *et al.* (1987) also found that GD sterility could be enhanced by the addition of Birmingham chromosomes. Moreover, GD sterility is temperature dependent in the same direction as pupal lethality. The phenotype of the GD sterility we observe from the Δ2-3/Birmingham combination is indistinguishable from that of standard P-M hybrid dysgenesis, at least on the level of gross morphology. The only difference is that the Δ2-3/Birmingham combination seems to be more severe at lower temperatures. This difference might be merely a consequence of the exceptionally high levels of transposase activity and the lack of repressor in Δ2-3, as opposed to ordinary P strains which make both repressor and transposase (H. ROBERTSON and W. ENGELS, unpublished data). We propose that both GD sterility and pupal lethality can be explained by a common cytological mechanism, differing only in which tissues are affected.

Cytological mechanism: To explain both pupal lethality and GD sterility, we suggest that the Δ2-3/Birmingham combination produces cell-lethal events, probably chromosome breaks, in both germ and somatic cells. These events require the Δ2-3 element to produce transposase, and the nonautonomous Birmingham elements to serve as substrate. Pupal lethality results from the accumulation of such events in the imaginal discs and histoblast cells during larval development. This model successfully explains the absence of lethality during larval stages where the cells necessary for life are mostly polytene and nondividing, and therefore tolerant of chromosome breakage. Meanwhile, cell-lethal events in the imaginal disks and histoblasts will occur, but cause no harmful effects until the time of pupariation when evagination of imaginal discs takes place and when cell division begins in histoblasts. Our finding that the temperature sensitive period occurs during the three larval instars is in good agreement with this model. Moreover, ionizing radiation applied to larvae has been observed to produce pupal lethality (WADDINGTON 1942; VILLEE 1946; POSTLETHWAIT and SCHNEIDERMAN 1973; POSTLETHWAIT 1975; HAYNIE and BRYANT 1978). The postulated mechanism is chromosome breakage and cell death in the imaginal discs followed by compensatory growth of surviving cells. When irradiated larvae survive to adulthood, they tend to have patches of missing tissue and duplicated segments similar to those we observe in the survivors of the Δ2-3/Birmingham combination. Finally, radiation-induced pupal lethality is much more frequent in males than in females (OSTER and CICAK 1958). This observation parallels our finding that when Δ2-3 is present the Birmingham *X* chromosome is more often lethal in the hemizygous condition than when it is heterozygous in females.

Similarly, according to this model, GD sterility would be explained by cell-lethal events in the germline, at least some of which must be dominant. Such events would have to occur at extremely high frequencies (ENGELS 1983) to explain the observed levels of GD sterility seen in some crosses. To explain the dependence upon temperature of both pupal lethality and GD sterility, we postulate that chromosome breaks produced by *P* element activity are repaired less efficiently at elevated temperatures. In fact, various results in both plants (SAX and ENZMANN 1939) and animal cells (BAJERSKA and LINIECKI 1969) have indicated that DNA repair following radiation damage is less effective at higher temperatures. Another possibility is that the production or activity of transposase made by Δ2-3 is temperature dependent. However, previous work indicates that other dysgenic traits, particularly sn^w hypermutability (ENGELS 1979a, 1981), show temperature effects that are much less pronounced than the ones we see for GD sterility and pupal lethality. The difference, we suggest, is that the DNA repair mechanisms involved in sn^w mutability and other dysgenic traits are different from those of GD sterility and pupal lethality, the latter being more temperature sensitive.

SIMMONS *et al.* (1987) have recently proposed a model for GD sterility that is similar to the above, but it is based on different kinds of evidence. Experiments are in progress to test this hypothesis for pupal lethality using cytological methods.

Evolutionary considerations: It is not known whether the *P* element's germline-specific splice reflects a general mechanism for limiting the expression of certain genes to the germline, or whether it is specific to *P* elements (LASKI, RIO and RUBIN 1986). Either way, we can assume that natural selection has acted to favor a *P* element sequence that is mobile only in the germ line. Such selection might have been applied directly on the transposable elements or indirectly on the organism (ENGELS 1986).

If *P* elements are assumed to be parasitic in their interactions with the host organism, then selection acting on the elements will favor those that do the least damage to their hosts, provided the elements can still transpose. CROW (1984) pointed out that there is an evolutionary advantage to confining transposition to the germline since somatic activity is likely to be harmful to the organism without providing any benefit to the element. Germline transposition, unless it produces complete sterility, permits the element to spread to unaffected individuals. The present results

are in good agreement with this notion, since they show that somatic *P* element activity can be highly deleterious to the organism.

The taxonomic distribution of *P* elements suggests that they have only existed in the *Drosophila melanogaster* genome since the species diverged from its various sibling species (BROOKFIELD, MONTGOMERY and LANGLEY 1984, DANIELS *et al.* 1984; LANSMAN *et al.* 1985), which could have been as recently as one million years ago. One author (KIDWELL 1979, 1983) proposed that *P* elements may have first appeared within the current century. In that case, we must assume that their germline specificity evolved in another species but continued to function when *P* elements arrived in *D. melanogaster*. On the other hand, if *P* elements appeared on the order of a million years ago, then the severity of the consequences we observe for somatic activity is sufficient that a tissue specific intron could well have evolved entirely within *D. melanogaster*.

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APPENDIX: THE MODEL OF INDEPENDENT ACTION

Suppose each of the major Birmingham chromosomes has a fixed probability of causing pupal lethality in conjunction with Δ2-3, and this probability is not dependent on which other Birmingham chromosomes are present. Then if two Birmingham chromosomes are present, say the second and third, then the probability of pupal death would be $1 - (1 - \theta_2)(1 - \theta_3)$, where θ_2 and θ_3 are the probabilities of lethality caused by the second and third chromosomes, respectively.

Let φ_i be the relative recovery frequency of the ith Birmingham chromosome. It will be proportional to $1 - \theta_i$, with the proportionality constant equal to the reciprocal of the recovery frequency of the non-Birmingham homolog if the non-Birmingham chromosomes are assumed to have a recovery frequency of unity. Then we can think of the φ_i's as survival probabilities. According to the model, the data in Table 1 would come from a multinomial distribution with indexes:

$$\frac{1}{D_1}, \frac{\varphi_1}{D_1}, \frac{\varphi_2}{D_1}, \frac{\varphi_3}{D_1}, \frac{\varphi_1\varphi_2}{D_1}, \frac{\varphi_1\varphi_3}{D_1}, \frac{\varphi_2\varphi_3}{D_1}, \frac{\varphi_1\varphi_2\varphi_3}{D_1}$$

corresponding to the categories in the table from left to right. D_1 is the sum of the numerators. Similarly, Table 2 is considered as a multinomial distribution with indexes:

$$\frac{1}{D_2}, \frac{\varphi_2}{D_2}, \frac{\varphi_3}{D_2}, \frac{\varphi_2\varphi_3}{D_2}$$

where $D_2 = 1 + \varphi_2 + \varphi_3 + \varphi_2\varphi_3$.

If the observed counts in Table 1 for any given row are designated $n_1, n_2, \ldots, n_8$, $(N = \Sigma n_i)$, and those of the corresponding row of Table 2 are designated m_1, m_2, m_3, m_4, $(M = \Sigma m_i)$, respectively from left to right in both cases, then the likelihood ratio for the experiment is the product of two multinomials:

$$LR(\varphi_1, \varphi_2, \varphi_3) = \left[\binom{N}{n_1 n_2 \ldots n_8}\left(\frac{1}{D_1}\right)^{n_1} \cdots \left(\frac{\varphi_1\varphi_2\varphi_3}{D_1}\right)^{n_8}\right] \times \left[\binom{M}{m_1 m_2 m_3 m_4}\left(\frac{1}{D_2}\right)^{m_1} \cdots \left(\frac{\varphi_2\varphi_3}{D_2}\right)^{m_4}\right].$$

Taking the log, eliminating constant factors, and simplifying shows that the maximum likelihood estimates of the φ_i are obtained by maximizing the quantity:

$$a_1 \ln \varphi_1 + a_2 \ln \varphi_2 + a_3 \ln \varphi_3 - N \ln D_1 - M \ln D_2$$

with respect to φ_1, φ_2 and φ_3, where the constants a_i are the observed numbers of survivors with the ith Birmingham chromosome. Thus:

$$a_1 = n_2 + n_5 + n_6 + n_8$$
$$a_2 = n_3 + n_5 + n_7 + n_8 + m_2 + m_4$$
$$a_3 = n_4 + n_6 + n_7 + n_8 + m_3 + m_4.$$

Numerical maximization using the simplex algorithm of NELDER and MEAD (1965) was carried out for each temperature and both crosses to produce the estimates in Table 3. The expected numbers in Tables 1 and 2 came from multiplying the totals for each row by the appropriate frequency, obtained by substituting the maximum likelihood estimates of the φ_i in the multinomial indexes shown above.