Hybrid dysgenesis in *Drosophila melanogaster*: a new type of gonadal dysgenic sterility expressed at the pre-pupal stage

Fuyuo HIHARA

*Laboratory of Biology, Faculty of General Education,
Ehime University, Matsuyama, Ehime 790, Japan*

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ABSTRACT

Two sublines, B-202 and B-207, which were derived from crosses between Or-R (M) females and *Cy/Pm* (P) males were found to cause a new type of gonadal dysgenic sterility, designated as the GD-3. GD-3 sterility showed a typical reciprocal cross effect under the P-M system. It was caused at the frequency close to 100% in dysgenic offsprings reared above 25°C, of which gonads were morphologically clearly different from those of usual GD sterility, whereas there was no indication of GD-3 sterility at temperatures below 24°C. Temperature sensitive period of GD-3 sterility was estimated to the prepupal stage by shift-down experiment. In the B-202 subline, the 2nd chromosomes marked with *Pm* alone carried GD-3 elements. Those of the B-207 subline, however, were estimated to locate both on the 2nd and 3rd chromosomes, acting synergistically with each other.

1. INTRODUCTION

Germ line abnormalities that are induced in hybrid offsprings derived from the crosses between M females and P males are brought about by activation of P transposable elements (Kidwell et al., 1977). P strains have many P elements which are heterogeneous in size and are dispersed throughout the genome, whereas M strains completely lacked them (O'Hare and Rubin, 1983).

P elements are activated exclusively under the M cytotype, a maternally transmitted cytoplasm state from M strain (Engels, 1979a, b). P element activity is repressed under the P cytotype determined by P element encoded repressor. The mobility of P elements in M cytotype usually occurs only in the germ line. This restricted nature is regulated at the RNA splicing level (Laski et al., 1986; Rio et al., 1986).

Most of the hybrid dysgenesis, such as high mutability, male recombination and chromosome breakage, is due to mobility of P elements. The molecular basis of gonadal dysgenic (GD) sterility, however, is not clearly understood so far. For one possible mechanism of GD sterility, Engels et al. (1987) proposed that it might be caused by P element-mediated chromosome breakage. Recently, Margulies(1990) found a kind of gonadal dysgenesis which was caused in both

sexes at the low (21°C) restrictive temperature using a subline of the Harwich P strain. This gonadal dysgenesis was strengthened by meiotic mutant genes. Thus, DNA repair system might be essential for the repair of lesions induced by P element transposition.

This paper reports another kind of gonadal dysgenic (GD) sterility which is expressed at the temperatures above 25°C in both sexes. The temperature sensitive period of this sterility is much later than that of usual GD sterility; it takes about 108 hr after oviposition (the pre-pupal stage) at 27°C.

In dysgenic hybrids which were reared higher than 25°C, the germ cells developed to the spermatocytes in males and to the germarium formation stage in females.

2. MATERIALS AND METHODS

Drosophila stocks: All genetic symbols not otherwise explained are in Lindsley and Grell (1968). The stocks used as M strains for assessing GD sterility are *Oregon-R*, *Canton-S*, *C(1)DX,y,f*, *rucuca/TM-3*, *ebony* and *y sn^w;bw;st*. The last mutant stock carries a P element insertion at the *singed* locus but behaves as M strain having no repressor effects (Engels, 1979b; Roiha et al., 1988).

The original P strain, *Cy/Pm (P)*, has been described previously (Hihara et al., 1985). B-202 P type subline was established from pair matings between *Or-R* female and *Cy/Pm (P)* male at 25°C, each single F₁ *Cy* or *Pm* male in a vial being backcrossed for five generations to *Or-R* females. In subsequent ten generations, both sexes with *Cy/+* or *Pm/+* genotypes were selected as parents and maintained by small mass mating. At the 16th generation, diallele crosses between these inbred lines were performed to measure the allelic rate of the lethal or sterile mutant genes on the X and 3rd chromosomes. In the course of this experiment, B-202 subline was found to have an ability inducing unusual male sterility. B-207 subline was derived from crosses with the same procedure as in B-202, except that P strain-derived 2nd chromosomes were removed from the genome at the 16th generation.

Both B-202 and B-207 sublines are typical P type; they can cause *sn^w* secondary mutation and GD sterility when crossed with M type females, and in reciprocal crosses, repress these dysgenic traits. Another P strain used was π_2 which was fully documented by Engels (1979b).

For mapping GD elements of B-207 subline, a balancer stock, *Cy/Pm;Sb/Ubx* and a stock with multiply marked 2nd chromosome, *S*, *Sp*, *Bl*, *L*, *bw^D* were used. They were typical M type stocks. Mating schemes using these stocks to map GD elements of B-207 subline will be presented later in the experiments to obtain corresponding results.

GD sterility measurement: GD sterility was usually measured by the frequency of dysgenic gonads in the flies aged 2–4 days after emergence. Those shown in

Figure 7, however, were determined by scoring flies with at least unilateral dysgenic gonads as to be sterile to get more sensitive information on the location of GD elements on the 2nd chromosomes.

Terminology: It is convenient to distinguish three types of GD sterility in the PM system, including that described in this paper. The usual GD sterility having the highest restrictive temperature of 29°C to induce male sterility is designated as GD-1 sterility. That of the lowest restrictive temperature of 21°C to induce male and female sterility as reported by Margulies (1990) is designated as GD-2, and the third type having intermediate restrictive temperature as GD-3 sterility which will be described here.

3. RESULTS

Temperature sensitive sterility in hybrids derived from crosses between M females and P males.

Males of P type subline, B-202, originated from a cross between Oregon-R(M) females and *Cy/Pm* (P) males were found to induce sterility in hybrid males when they were crossed to M females. The most prominent difference of this GD-3 sterility from GD-1 is that it lowered restrictive temperature in males; they expressed high frequency of GD-3 sterility at 25°C. The original *Cy/Pm* strain has no ability to induce GD-3 sterility in hybrid males when it was crossed to M females.

As shown in Table 1, F₁ *Pm* males derived from B-202 subline fathers showed

Table 1. Temperature sensitive GD-3 and GD-1 sterility in F₁ hybrids derived from the B-202 strain crosses (27°C)

Cross			Male GD-3 sterility				Female GD-1 sterility			
family	male		<i>Pm</i>		<i>Cy</i>		<i>Pm</i>		<i>Cy</i>	
			n	%s	n	%s	n	%s	n	%s
<i>rucuca/TM-3</i>	B-202(<i>Cy/Pm</i>)	M×P	200	98.5	200	0.0	200	100.0	200	98.0
<i>Canton-S</i>	B-202(<i>Cy/Pm</i>)	M×P	200	100.0	200	0.0	200	99.0	200	100.0
<i>C(1)DX, yf</i>	B-202(<i>Cy/Pm</i>)	M×P	200	98.0	200	0.0	200	98.0	200	99.0
<i>ebony</i>	B-202(<i>Cy/Pm</i>)	M×P	200	96.7	200	0.0	200	100.0	200	98.5
<i>y sn^w</i>	B-202(<i>Cy/Pm</i>)	M×P	200	100.0	200	0.0	200	97.8	200	97.8
B-202(<i>Cy/Pm</i>)	<i>rucuca/TM-3</i>	P×M	200	0.0	200	0.0	200	2.8	200	1.3
B-202(<i>Cy/Pm</i>)	<i>Canton-S</i>	P×M	200	0.0	200	0.0	200	0.0	200	2.3
B-202(<i>Cy/Pm</i>)	<i>C(1)DX, yf</i>	P×M	200	0.0	200	0.0	200	5.0	200	2.3
B-202(<i>Cy/Pm</i>)	<i>ebony</i>	P×M	200	0.0	200	0.0	200	1.3	200	2.8
B-202(<i>Cy/Pm</i>)	<i>y sn^w</i>	P×M	200	0.0	200	0.0	200	4.3	200	1.0

n: number of flies observed.

%S: % sterile testis or ovary.

nearly complete GD-3 sterility when they were developed at 27°C, regardless of female parent of M strains mated. F₁ *Cy* males, however, did not show any tendency of GD-3 sterility. In reciprocal crosses of the same sets of parents, no GD-3 sterility was found both in *Pm* and *Cy* sons, showing that GD-3 sterility was repressed by the maternally contributed P cytotype like as GD-1 sterility.

The right two columns of Table 1 give the frequency of GD-1 sterility scored for hybrid females. In contrast to male GD-3 sterility, both *Pm* and *Cy* females showed nearly complete GD-1 sterility at this non-permissive temperature. It was not detectable whether or not GD-3 sterility was also induced in hybrid females, because GD-1 sterility was preferentially expressed over GD-3 sterility as will be described later.

Thus, B-202 subline males have both usual P elements causing GD-1 sterility and elements inducing GD-3 sterility, GD-3 elements in B-202 subline being preferentially associated with *Pm* chromosomes. Most of the other P type sublines derived from similar parental crosses as B-202 did not have ability to induce GD-3 sterility except for B-207 subline which will be described later. This suggests that GD-3 elements could not be obtained simply by crossing Oregon-R females to *Cy/Pm* males.

It is important to test whether GD-3 sterility found for B-202 subline is a general feature of P type strain as opposed to being a special property of GD-3 elements of B-202 subline.

Table 2 represents the results obtained from a similar series of crosses using π_2 strain which has been well known to be strong P type. The results showed that males of π_2 strain also induce GD-3 sterility in F₁ males reared at 27°C, though the frequency was 10–13% which was much lower than that of B-202 subline. Again, in this case, GD-3 sterility in F₁ males derived from the reciprocal cross, between

Table 2. Temperature sensitive GD-3 sterility in F₁ hybrids derived from the π_2 strain crosses (reared at 27°C)

Cross			GD-3 sterility (male)*					GD-1 sterility (female)*				
female	male		2	1	0	T	%S**	2	1	0	T	%S**
<i>rucuca/TM3</i>	π_2	M×P	160	28	12	200	13.0	0	0	200	200	100
<i>Canton-S</i>	π_2	M×P	164	30	6	200	10.5	0	0	200	200	100
<i>C(1)DX, yf</i>	π_2	M×P	165	26	9	200	11.0	0	0	200	200	100
<i>y sn^w</i>	π_2	M×P	162	25	13	200	12.8	0	0	200	200	100
π_2	<i>ebony</i>	P×M	200	0	0	200	0.0	200	0	0	200	0

*2; flies with a pair of normal testes or ovaries.

1; flies with a pair of normal and unilaterally dysgenic testes or ovaries.

0; flies with a pair of bilaterally dysgenic testes or ovaries.

T; total number of flies examined.

**% sterile testis or ovary.

π_2 female and *ebony* males, was completely repressed. The behavior of the π_2 strain is essentially identical with B-202 subline regarding to induction of GD-3 sterility. This suggests that GD-3 sterility is not restricted to B-202 subline but a general property of P type strain.

Morphological features of the GD-3 sterility.

Dysgenic *Pm* males of B-202 fathers when they were reared at 27°C showed the GD-3 type testes; the length of them was about 1/3 compared to that of *Cy* brothers which had normal testes under the same rearing temperature (Fig. 1d). Arrest of spermatogenesis of such *Pm* males was observed just before the meiotic stage in these reduced testes. There are many encysted spermatogonia and nearly matured spermatocytes, the latter of which were characterized by connected filamentous mitochondria around the nucleus. Cysts undergoing meiotic divisions or having elongated spermatids, however, were not found at all.

Both *Pm* and *Cy* males raised at 29°C showed GD-1 type testes; the length of these testes was much shorter than those of GD-3 type and spermatogenesis failed to succeed beyond unencysted spermatogonial stage (Fig. 1a). A part of dysgenic *Pm* males reared at 28°C frequently had the paired GD-1 and GD-3 type testes (Fig. 1c). In contrast to this, *Pm* males which were cultured at 25°C sometimes had the paired GD-3 and normal testes. No GD-3 type testis was found in males when they were cultured under 24°C.

F₁ *Pm* males raised at 27°C were transferred to the new vials and were kept at 20°C for five days to observe whether or not the spermatogenesis was recovered. Since these males resulted in the same sterility rate as compared with males kept at 27°C for the whole period, GD-3 sterility should not be recovered by low temperature treatment after emergence. Thus, GD-3 sterility, which was induced in *Pm* males of B-202 fathers, was morphologically distinguishable from GD-1 sterility by means of whole testis length and arrested stage of spermatogenesis.

In dysgenic *Pm* females, it was not clear, by simple temperature treatment as in males, whether GD-3 sterility was induced; GD-3 sterility in females was usually covered up by GD-1 sterility having the same permissive temperature threshold as GD-3. Dysgenic *Pm* females with only GD-3 sterility were obtained, however, by temperature shift from the permissive (23°C) to the restrictive (27°C) state after the sensitive period of GD-1 sterility. The GD-3 ovaries were larger than those of GD-1 (Fig. 1f). In the ovarioles of the GD-3 ovaries, oogenesis ceased at the germarium stage (Stage 1 by King, 1970); there was no ovariole with the previtellogenic or the vitellogenic stages. More precise observation showed that GD-3 ovarioles had cystocytes in their germaria, in contrast to GD-1 ovarioles in which had only a smaller number of unencysted oogonia. These observations on GD-1 ovaries were in good agreement with those described by Schaefer et al. (1979).

Fig. 1. The gonads of the dysgenic flies from the cross, *ebony* females x B-202 males. (a) A pair of normal testes (arrow heads) of the *Cy* flies reared at 27°C. The bar represents 200 μ m. (b) A pair of GD-1 type testes (arrow heads) of the *Cy* flies reared at 29°C. (c) The GD-3 type (left arrow head) and GD-1 type (right arrowhead) testis of the *Pm* flies reared at 28°C. (d) A pair of GD-3 type testes of the *Pm* flies reared at 27°C. (e) A pair of GD-1 type ovaries of the *Cy* flies reared at 27°C. (f) A pair of GD-3 type ovaries of the *Pm* flies which rearing temperature was shifted from 23 to 27°C at the 2nd instar larvae.

Temperature sensitivity of GD-3 sterility.

To measure a more precise temperature sensitivity of GD-3 sterility, *ebony* females were crossed with B-202 males. Cultures were concurrently set up at different temperatures ranging from 21 to 29°C. Emerged F₁ males and females

Fig. 2. Temperature sensitivities of GD-3 and GD-1 sterility.

were aged separately for 2–3 days allowing gonadal development before dissection.

Figure 2 shows the temperature sensitivity of GD-3 sterility together with that of GD-1. The frequencies of female GD-3 sterility above 24°C were obtained after performing shift of the rearing temperature from 23°C to each indicated temperature at the stage of the 2nd instar larvae. Temperature sensitivity of GD-3 sterility was similarly appeared both in *Pm* males and females; virtually 100% sterility was induced at 26–29°C. In contrast, *Cy* males and females which were reared simultaneously with *Pm* flies did not show GD-3 sterility for all range of temperatures treated. *Pm* flies raised below 24°C consistently showed no GD-3 sterility, and those reared at 25°C showed variable frequencies of GD-3 sterility among replicate cultures, ranging from 47 to 89%. These results indicate a characteristic feature in temperature sensitivity of GD-3 sterility that restrictive threshold lies similarly at 25°C in both sexes.

Temperature sensitive period of GD-3 sterility.

The temperature sensitive period of GD-3 sterility was determined; the GD-3 type testes were scored for a series of *Pm* males of which rearing temperature was shifted down from 27°C to 21°C at every 24 hr after the oviposition (Fig. 3a). The results implies that GD-3 sterility was caused by approximately 108-hrs-development after the oviposition. Developmental stage of this sensitive period corresponded to the Stage P1 (white puparium) presented by Bainbridge and Bownes (1981) for the staging of living materials.

The sensitive period of GD-1 sterility scored for *Cy* females derived from the same parents as *Pm* males was much earlier than that of GD-3 sterility. As

Fig. 3. Comparison of the temperature sensitive period between GD-3 (a) and GD-1 (b) sterility. Both sterilities were determined by scoring GD-3 type testes in *Pm* males and GD-1 type ovaries in *Cy* and *Pm* females, respectively.

shown in Figure 3b, GD-1 sterility was caused from 6 to 24 hr (15 hr on average) after the oviposition when the rearing temperature shifted down from 27°C to 21°C. The sensitive period of GD-1 sterility obtained here was similar to that of the previous work (Kidwell and Novy, 1979).

Thus, the temperature sensitive period of GD-3 sterility is much later than that of GD-1 sterility, the former being the prepupal and the latter the embryonic stage. The difference of the temperature sensitive period between GD-1 and GD-3 sterility well corresponded to the morphological differences of the gonads in the flies reared at restrictive temperature; the spermatogenesis ceased at the unencysted spermatogonia in GD-1 sterility of which temperature sensitive period was at the embryonic stage and it advanced to the mature spermatocytes in GD-3 sterility having the temperature sensitivity at the prepupal stage.

Mapping GD-3 elements in the B-202 and B-207 sublines.

GD-1 sterility has supposedly been caused synergistically by P element copies dispersed throughout all the chromosomes. In the B-202 subline, however, GD-3 elements were preferentially linked with the *Pm* chromosome. To assess whether GD-3 elements are located on the *Pm* chromosomes alone or both major autosomes, the mating was performed as shown in Fig. 4. The data given in Table 3 show that the frequency of GD-3 sterility in $Pm^{202}/+;e/e$ males was not reduced compared to that of $Pm^{202}/+;B-202/e$ males. This suggests that GD-3 sterility was sufficiently caused when the *Pm* chromosome was introduced into the genome of F_2 male, regardless of the constitution of the 3rd chromosomes.

The *Pm* chromosome of B-202 subline carries inversions with multiple-break

Fig. 4. Mating scheme for detecting the B-202 chromosomes on which GD-3 elements locate. Hatched bars represent the 2nd and 3rd chromosomes of the B-202 subline, the dashed bars represent the *ebony* chromosomes.

Table 3. Effects of B-202 derived major autosomes on GD-3 sterility in males reared at 27°C

Genotype of F2 male	$Pm^{202}/+;B-202/e$	$Pm^{202}/+;e/e$	$+/+;B-202/e$	$+/+;e/e$
Number of F2 males tested	200	200	200	200
% GD-3 sterility	100	100	0	0

Pm^{202} ; B-202 derived 2nd chromosome marked with Pm .

B-202; B-202 derived 3rd chromosome

rearrangements which prevent mapping of GD-3 elements which are fully restricted to the Pm chromosome. More extensive search revealed that B-207 subline, which was established simultaneously with B-202, had ability to induce GD-3 sterility. The second chromosomes of this subline were originally derived from Oregon-R and, accordingly, had no inversion in them. Hybrid males between B-207 males and ebony females, when they were raised at 27°C, showed GD-3 sterility with 70–95% frequencies according to 15 replicate cultures of this subline. In subsequent experiments, flies of a culture which could give rise to the highest frequency (95%) of GD-3 sterility were used.

To determine the location of GD-3 elements among major autosomes of the B-207 subline, matings shown in Fig. 5 were performed. As summarized in Table

Fig. 5. Mating scheme for detecting the B-207 chromosomes on which GD-3 elements locate. Solid and reticulated bars represent the chromosomes of $Cy/Pm;Sb/Ubx$ strain and the B-207 subline, respectively.

Table 4. Effects of B-207 derived major autosomes on GD-3 sterility in males reared at 27°C

Genotype of F2 male	<i>B-207/+;B-207/e</i>	<i>B-207/+;Ubx/e</i>	<i>Cy/+;B-207/e</i>	<i>Cy/+;Ubx/e</i>
Number of F2 males tested	171	205	196	311
% GD-3 sterility	96.5	2.2	16.1	0.2

B-207; B-207 derived 2nd and 3rd chromosomes.

4, F₂ males having both major autosomes of B-207 heterozygously exhibited GD-3 sterility with 96.5% frequency at 27°C. However, the GD-3 frequencies in males heterozygous for the B-207 either in the 2nd (*B-207/+;Ubx/e*) or the 3rd (*Cy/+;B-207/e*) chromosomes were much lower, being 2.2 and 16.1%, respectively.

As the combination of the B-207 autosomes caused GD-3 sterility more effectively than the corresponding individual chromosomes, each B-207 autosome carries GD-3 elements acting synergistically to cause GD-3 sterility, being opposite to the B-202 subline of which GD-3 elements were restricted to the 2nd chromosome.

To map GD-3 elements on the second chromosomes of the B-207 subline, mating was performed as shown in Fig. 6. As indicated in Table 4, it was supposed that the 2nd chromosomes of B-207 alone could not be effective in causing GD-3 sterility. The frequencies of GD-3 sterility among the recombinants therefore were scored in the presence of the B-207 3rd chromosomes heterozygously to obtain an indication whether a part(s) of the B-207 2nd chromosome were responsible for GD-3 sterility.

The results are in Fig. 7. Among 11 recombinants, six showed GD-3 sterility substantially above the mean frequency of the control flies whose genotype was *+/+;B-207/e*, showing 26.0% GD-3 sterility. Four, (*S*), (*bw^D*), (*L,bw^D*), (*S,bw^D*), out of six recombinants had the *Sp⁺-Bl⁺* region of the B-207 and the rests, (*S,Sp*) and (*Sp*), were possible to have a part of this region through recombination between the *Sp⁺* and *Bl⁺* genes. These results suggested that GD-3 elements with major effect might be located within this region, though the exact copy number was not known.

Another trait associated with GD-3 elements among the recombinants was that (*bw^D*), (*L,bw^D*), and (*S,bw^D*) flies showed especially high frequency of GD-3 sterility. All of these recombinants had or possibly had the *S⁺-Sp⁺* region of the B-207 chromosome. This suggested that another GD-3 elements might be involved in this region. The most conspicuous difference of the GD-3 frequency related to this region was found between (*S,Sp,bw^D*) and (*S,bw^D*) flies, the former giving 28.1% and the latter 75.0% sterility. (*Sp,Bl,L,bw^D*) flies did not induce GD-3 sterility above the background level as revealed by mean GD-3 frequency of the flies having B-207 3rd chromosome heterozygously. This suggested that the *S⁺-Sp⁺* region alone could not increase GD-3 frequency when the *Sp⁺-Bl⁺* region

Fig. 6. Mating scheme for mapping GD-3 elements on the 2nd chromosome of the B-207 subline. Chromosomes are represented as in Figure 5 except that solid bars are used also for chromosomes of the S, Sp, Bl, L, bw^D strain.

of the B-207 chromosome was not included simultaneously.

Results of GD-1 sterility obtained from females suggested that GD-1 elements with pronounced effects might be located between the Bl^+-L^+ genes of the B-207 chromosome. Three types of recombinants, (S, Sp, Bl, L) , (Bl, L, bw^D) , and (Sp, Bl, L, bw^D) , had not accepted the Bl^+-L^+ region of the B-207 chromosome and their GD-1 frequencies were lower than or nearly same as the background level (40.7% sterility).

Thus, GD-3 and GD-1 elements were supposed to be differently distributed along the B-207 2nd chromosome. To confirm this, Spearman's rank correlation coefficient was calculated between the frequencies of GD-3 and GD-1 sterilities among 11 recombinants. The value obtained ($r=0.400$), however, was not significantly different from $r=0.0$ ($p>0.05$). This means that GD-3 and GD-1 elements are differently distributed among regions of the 2nd chromosomes of the B-207 subline.

Fig. 7. The effects of the recombinant 2nd chromosomes of the B-207 subline on GD-3 and GD-1 sterility. Reticulated and hatched bars represent the regions derived or possibly derived from the B-207 subline, respectively. Solid bars represent the chromosome regions of the *S*, *Sp*, *Bl*, *L*, *bw^D* strain. See the text for details.

4. DISCUSSION

Is GD-3 sterility caused by P elements?

GD-3 sterility have common characteristics with another types of GD sterility. First, sterility is caused with close to 100% in dysgenic flies raised at restrictive temperatures. And, in reciprocal crosses, sterility is completely repressed even in restrictive temperatures, resulting from a regulation mechanism known as P cytotype. Second, there is an accurate developmental timing to cause temperature sensitive gonadal dysgenesis. Third, temperature difference between restrictive and permissive state is so small that sterility is induced in all or nothing

fashion. These common natures among GD sterilities strongly suggest that GD-3 sterility may also be caused by P elements.

Morphologically, the gonads of GD-3 flies were resemble to those caused by *hobo* elements (Yannopoulous, 1978). Induction of GD sterility by *hobo* elements, however, is not the same as that of the reciprocal cross effects of the P-M system (Stamatis et al., 1989). In contrast, GD-3 sterility was caused completely under this rule as presented in Tables 1 and 2. Moreover, the temperature dependence of GD sterility caused by *hobo* elements was clearly distinct from those caused by P elements; the dysgenic gonads by *hobo* elements were induced at a temperature as low as 14°C, and the percentage of the dysgenic gonads increased linearly up to 25°C, followed by dramatic reduction to approximately 20% at 29°C. As shown in Fig. 2, temperature sensitivity of GD-3 sterility was quite different from that obtained by *hobo* elements.

Temperature sensitivity and temperature sensitive period of GD-3 sterility.

GD-3 sterility is different from the other sterilities in two properties; temperature threshold is 25°C in both sexes and temperature sensitive sterility occurs in prepupal stage (Fig. 3a).

The fact that there are differences of restrictive temperature among GD sterilities itself can not be an evidence that GD-3 sterility is not caused by P elements. In fact, GD-2 sterility of which restrictive temperature is nearly at 21°C was found in hybrid offsprings originated from crosses of a subline of Harwich P line. Engels et al.(1987) also found that GD sterility was induced at lower temperature than GD-1 sterility when nonautonomous P elements derived from Birmingham M' strain were mobilized by a modified P element $\Delta 2-3(99B)$ which was used as a source of P transposase.

GD-3 sterility as estimated by temperature sensitive period occurred both in males and females at a short period of prepupal stage and differentiation of the germ cells was blocked at a special stage of the spermatogenesis or oogenesis. Thus, GD-3 sterility was caused at a temporally and spatially limited range, as in GD-1 sterility which temperature sensitive period was at the embryonic stage (Fig. 3b).

On the other hand, Engels et al. (1987) found that temperature sensitivity of GD sterility continued throughout the larval development. This sterility caused by combination of the Birmingham and the $\Delta 2-3$ bearing chromosomes seemed to have different features concerning to temperature sensitivity and temperature sensitive period as compared to the ordinary $M \times P$ crosses.

Dysgenic traits other than GD sterility, such as mutation induction and chromosome rearrangement can be related directly to P element mobility (Engels and Preston, 1984; Cooley et al., 1988). These traits, however, have no such a prominent temperature effect as in GD sterility. Accordingly, temperature sensitivity found in GD sterility can not be due to temperature dependence of the

synthesis or the activity of P transposase itself which is responsible for P insertion.

A possible mechanism of GD sterility.

As mentioned above, GD sterility has at least two common features. First, almost all dysgenic flies reared at higher developmental temperatures show GD sterility. Second, the arrest of the germ cell development in dysgenic flies is arose at a specific stage of gametogenesis.

If GD sterility is explained by P element insertional mutation, it must be postulated that P elements insert into genes required for germ cell development in all dysgenic flies. Insertional mutation rates measured so far, however, are neither so high nor so site specific as will be sufficient to explain properties of GD sterility (O'Hare and Rubin, 1983; Simmons et al., 1984). More recently, Bownes (1990), by using so called "enhancer-trap" methods, found a preference of P element insertions into genes being transcribed in the gonad. However, none of the insertions caused a mutation in a gene essential for male fertility. Thus, the possibility of insertional mutation as a cause of GD sterility can be ruled out.

The occurrence of GD sterility was positively correlated with the frequency of *sn^w* mutation (Engels, 1984; Kocur et al., 1986). Thus, both GD sterility and *sn^w* mutation have been regarded to be caused simply by the quantity of transposase. Rasmusson et al. (1990), however, failed to find a quantitative relationship between P element number and the incidence of gonadal dysgenesis. Transposase was estimated to have a helix-turn-helix structure which was thought to be characteristic for DNA binding protein (Rio et al., 1986). In fact, it interacts with P element specifically and also displays an unusually high non-specific affinity for DNA (Kaufman et al., 1989).

From these facts, a possible mechanism for GD sterility is hypothesized that P element proteins, such as transposase and its derivatives, might be bound thermo-dependently to genes essential for germ cell development and repress their expression. GD-3 and GD-2 elements might encode structurally different DNA binding proteins from those of GD-1 element, resulting that they recognize different genes in the gonad with different temperature sensitivity.

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