J Tokyo Wom Med Coll
66 (12) 1031～1044 (1996)

# THE PATTERN PROFILE OF *lac-Z* REPORTER GENE EXPRESSION IN ENHANCER TRAPPED STRAINS OF *DROSOPHILA MELANOGASTER*

**Etsuko MACHIYAMA** and **Akira KOMATSU**
Department of Physiology (Director: Prof. Yoko HASHIMOTO)
Tokyo Women's Medical College

(Received Aug. 16, 1996)

Patterns of expression of the reporter gene, *lac-Z*, were analyzed in 22 major organs of third instar larvae from a total of 101 independent enhancer trapped strains of the fruit fly, *Drosophila melanogaster*. The *lac-Z* gene expression showed diverse patterns among different organs and among different strains. Of the organs examined, the CNS showed the highest *lac-Z* expression (91% in the brain, 75% in the thoracico-abdominal ganglia), followed by imaginal discs (30% to 65%) and the digestive duct (30% to 50%), while muscles and oenocytes showed the lowest frequencies of *lac-Z* expression, at 13% and 11% respectively. Organs with high frequencies of gene expression may reflect a much more complex organ, consisting of many different cell types, such as the CNS, or it may reflect a dynamic developmental process under hormonal control in the morphogenesis of imaginal discs and in CNS reorganization. Many genes were expressed simultaneously in many different organs. The frequency of simultaneous gene expressions in different organs was spread over a wide range, from a few organs to almost all organs examined, and the average number of organs showing simultaneous gene expression was 4.4 both in the male and female. Simultaneous gene expression in a certain combination of organs was, in almost all combinations, higher than the simple product of the expression percentages of individual organs. These results indicate the presence of genes necessary for cellular processes shared by many organs, or necessary for basic cellular processes, such as housekeeping genes.

## Introduction

Enhancer trapping provides a useful method to identify, *in situ*, genes which express in spatially and temporally restricted patterns during development of the fruit fly, *Drosophila melanogaster* (*D. melanogaster*). It was certified that P-elements can transpose from extrachromosomal DNA to germ line chromosomal DNA in *D. melanogaster* embryos[1)2)]. O'Kane and Gehring[3)] developed an approach for *in situ* detection of genomic elements that regulate transcription in *D. melanogaster* through the insertion of a modified P-element carrying the *Escherichia coli* (*E. coli*) *lac-Z* coding region fused to a weak promoter into the *D. melanogaster* genome. If the insertion occurs near a transcriptional regulatory element, or enhancer, the pattern of *lac-Z* expression will mirror part, or all, of the transcriptional pattern of the gene normally associated with that regulatory element, as shown by histochemical staining of $\beta$-galactosidase ($\beta$-gal)[4)]. This method provides an effective way of analyzing direct determination of the gross pattern of gene expression in *D. melanogaster* during the developmental process.

In this paper, we report the characterization

of gene expression by analyzing the strains derived from insertion of an enhancer detector element which can be used to estimate the fraction of genome involved in a certain developmental stage. We examined 101 independent strains for the expression of *lac-Z* using histochemical localization of β-gal activity in the wandering stage of third-instar larvae. Examination of *lac-Z* expression patterns showed a remarkable diversity among organs and tissues, and among strains. The expression pattern was analyzed to get a profile of the P-insertions including the number of organs or tissues that expressed *lac-Z* in every strain, and conversely, the number of strains that expressed *lac-Z* in every organ and tissue. The results indicate that only few strains represented highly specific expressions of the *lac-Z* gene for a certain organ/tissue. The majority of strains expressed *lac-Z* in many organs simultaneously, and frequency of simultaneous *lac-Z* expression between two organs was, in most combinations, higher than expected by a simple product of both frequencies.

## Materials and Methods

### Strains

Flies were reared on a yeast-cornmeal-agar medium containing mold inhibitor. We used 101 fly strains harboring single P-element insertions in the autosomes (donated by Dr. D. Yamamoto, Mitsubishi-Kasei Institute of Life Sciences). The method for making P-insertion strains was described in Yokokura et al[5]. In brief, P-insertion strains were generated by jump-start mutagenesis[6], in which a P-lwB element on the X-chromosome was forced to translocate to an autosomal site by introducing a △2-3 P transposase source[7][8]. Mutations were first balanced with SM1 for second-chromosome insertions and with TM3 for third-chromosome insertions. After checking lethality, sterility and fertility, the fertile strains were kept as homozygotes. The P-lwB element contained the normal transposase promoter fused to the *E. coli* β-gal-encoding gene (*lac-Z*) as a reporter gene, the heat-shock-mini-*white* fusion gene as an eye color marker, and a Bluescript plasmid vector carrying an ampicillin resistance gene. Of 551 P-insertion strains, 17% were lethal, 4% were sterile, and the remaining 79% were fertile. We picked 101 strains randomly, for the present study, amongst the fertile strains. *Canton-S* was used as a wild type strain, *white*$^-$ ($w^-$) as a background control for P-insertion strains, and a $\beta$-*gal*$^{hl}$ strain as a β-gal-deficient strain[9][10].

### Staining for β-gal activity

Detection of *lac-Z* expression was performed as follows. A third-instar larva at the wandering stage was briefly washed with distilled water and was transferred to a small chamber containing physiological saline[11]. Then, the larva was pinned and cut open along the dorsal midline under a stereoscopic dissection microscope. The dissected larva was fixed for 15~20 min with 2 ml of fixative (0.75% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3), was washed twice with phosphate buffered saline (PBS)[12], and then was transferred into a microtiter plate well containing 800 μl of PBS and 0.3% polyoxyethylene (10) octylphenyl ether (Triton X-100). Meanwhile, staining solution[13] was prepared by preincubation of 0.5 m*l* aliquot of potassium ferricyanide and potassium ferrocyanide (Fe/NaP) solution (pH 7.2) adding 12.5 μl of 8% 5-bromo-4-chloro-3-indolyl-galactosidase (X-gal) in dimethyl sulfoxide (DMSO) at 37°C and incubated a further 10 min. Before use, the staining solution was centrifuged for 3 min to remove pellet crystals. The larva was incubated in the staining solution and was left at 37°C overnight. The larva was then washed three times with PBS containing 0.3% Triton X-100 to remove crystals generated during incubation. Photographs were taken using a stereoscopic dissection microscope, initially at low magnification. Fat bodies were eliminated if necessary for improved viewing. When a characteristic pattern of expression was observed in a certain organ or tissue, it was dissected out and transferred to a glass

slide. After photographs were retaken at a higher magnification, the specimen was mounted in PBS with a coverslip and sealed with nail varnish. To achieve optimal resolution of β-gal localization, we performed microscopic observation and photographs the day of mounting.

**Feulgen reaction**

To reveal nuclei, we stained *Canton-S* third-instar larvae with Feulgen reaction[14]. A dissected third-instar larva was fixed in 10% formalin, then immersed in preheated 3.5 N HCl at 37°C for 60 min to achieve optimal hydrolysis. After the specimen was rinsed with distilled water, it was immersed in Schiff's reagent for 2 h at room temperature and transferred three times to sulphite wash for 2 min to remove excess Schiff's reagent. After washing in distilled water, the specimen was photographed under a dissection microscope as in β-gal staining.

**Data processing**

We observed *lac-Z* expression in 22 organs and tissues, including the central nervous system (CNS), imaginal discs and digestive system. Figure 1 represents an illustration of the organs and tissues examined. When we quantified the *lac-Z* expression of different organs, we used an all-or-none grouping: we considered it a "positive (1)" expression when even a weak or par-

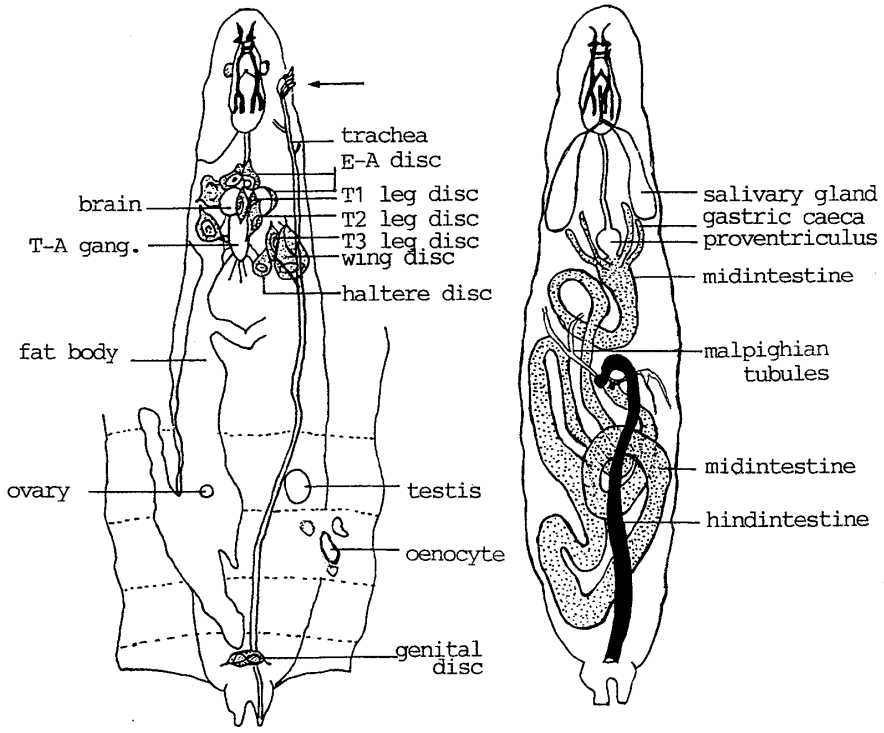
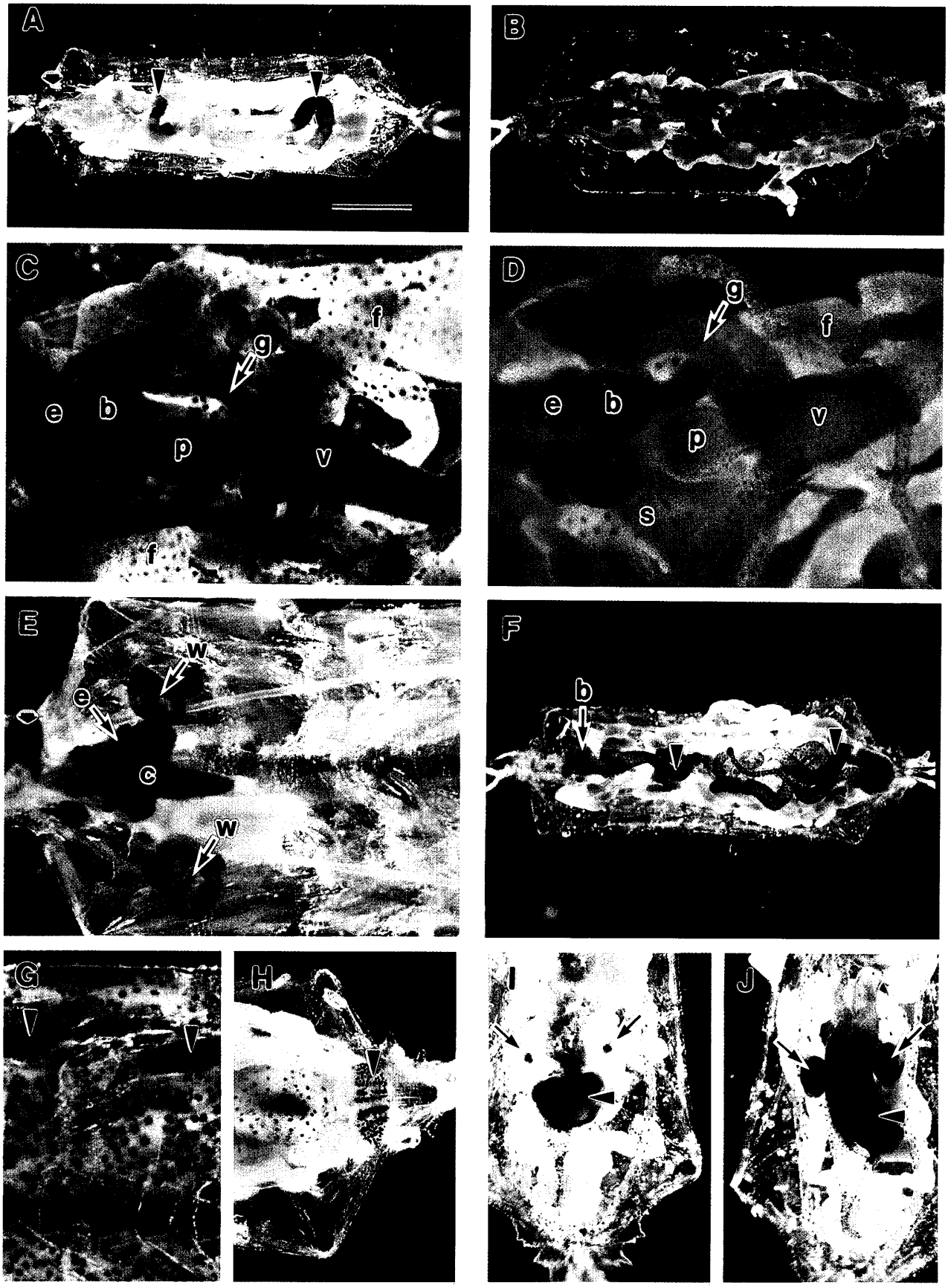



**Fig. 1** The organs and tissues in a third-instar larva of *Drosophila melanogaster*
Dorsal aspect; twenty two organs and tissues are illustrated for which *lac-Z* gene expression was examined.
Left: dissected internal organs other than the digestive system. Imaginal discs are drawn only on one side, and male and female gonads are drawn on the right and left sides respectively. A prothoracic tergite disc, which expresses the innate *lac-Z* gene, is indicated by an arrow.
Right: The digestive system, including the exocrine glands and digestive duct. Muscles and epidermis are omitted from these illustrations. Modified after Bodenstein[25].
Abbreviations: E-A disc, eye-antennal discs; Tl leg disc, prothoracic leg disc; T2 leg disc, mesothoracic leg disc ; T3 leg disc, metathoracic leg disc ; T-A gang., thoracico-abdominal ganglia.


A
B
C
f
g
e
b
p
v
f
D
g
f
e
b
v
p
s
E
w
e
c
w
F
b
G
H
I
J

tial *lac-Z* expression was detected, but "negative (0)" when no such expression was detected. After the *lac-Z* expression in 22 organs was put into binary code (1 or 0) for all 101 strains, data was analyzed by a personal computer (Apple Macintosh) using application software (Excel and Delta Graph).

## Results

### *lac-Z* Expression in control strains

As a background control, we examined *lac-Z* expression for the $w^-$ strain which had been used as a background to make P-element insertion strains. No *lac-Z* expression was observed in $w^-$ larvae except in three areas: anterior and posterior portions of the midintestine, and a pair of prothoracic tergite discs (Fig. 2A). The *Canton-S* larvae also showed *lac-Z* expression in these three local areas, suggesting that wild type larvae produce a native $\beta$-gal enzyme locally in these three areas. Such endogenous pattern of staining have been reported in embryos[3)15)]. To examine whether the *lac-Z* expression observed in these three areas of $w^-$ and wild type larvae was innate, we observed the *lac-Z* expression in a $\beta$-gal-deficient strain, $\beta$-$gal^{hl}$. None of the organs or tissues of this strain showed *lac-Z* expression (Fig. 2B). Thus, these three areas with presumptive innate *lac-Z* expression were omitted from further analysis. Reproducibility of the staining of *lac-Z* expression was quite good for each strain, with no difference in the staining pattern, except a slight difference in color depth.

### Localizaton of *lac-Z* product to the nucleus

The localization of $\beta$-gal within a cell has been reported to depend on the construct of the inserted P-element[16)]. When a P-element contains a *lac-Z* reporter gene fused to the P-element transposase gene (PZ element), this PZ

**Fig. 2** Sample photographs depicting *lac-Z* expression in several fertile strains and controls
**A**: *lac-Z* Expression in a $w^-$ strain as a background control which had been used as a background to make P-element insertion strains. No *lac-Z* expression was found except in anterior and posterior portions of midintestine (arrowheads) and the prothoracic tergite discs (short arrow). The *Canton-S* larva also showed a similar expression pattern. Whole dissected body.
**B**: *lac-Z* Expression in a $\beta$-$gal^{hl}$ strain which is deficient for innate *lac-Z* gene. Absolutely no *lac-Z* expression was found in this deficient strain. Whole dissected body.
**C** and **D**: Comparison between the $\beta$-gal staining (C) and Feulgen staining (D) in the anterior part of larval body: brain (b), eye-antennal discs (e), salivary gland (s), proventriculus (p), gastric caeca (g), ventriculus (v, anterior end of midintestine), and fat bodies (f). Stained cell nuclei are distinctly recognized from the cytoplasm in the digestive system and the fat body. Homogeneously stained organs such as the brain and imaginal discs may consist of small packed cells with large nuclei.
**E**: *lac-Z* Expression of a strain restricted to the CNS (c) and imaginal discs (wing discs, w; eye-antennal discs, e). The prothoracic tergite discs (short arrow) were also stained for innate *lac-Z* expression.
**F**: *lac-Z* Expression of a strain restricted to the digestive duct and the brain (b). The digestive duct was stained by dotted nuclei. Anterior and posterior portions of the midintestine (arrowheads) were also stained homogeneously for innate *lac-Z* expression.
**G**: *lac-Z* Expression in the epidermis of a strain. Fifth to sixth abdominal segments are shown. In this strain, all nuclei of the epidermal cells in the thoracico-abdominal segments were stained, as well as the oenocytes (arrowheads).
**H**: *lac-Z* Expression restricted to the epidermis of a segment. The sternite of the terminal abdominal segment (the eighth abdominal segment) was stained (arrowhead).
**I** and **J**: *lac-Z* Expression in the gonads of female (ovary; arrows in I) and male (testis; arrows in J). Posterior portions of midintestine (arrowheads) were also stained for innate *lac-Z* expression.
Anterior to the left in A to H. Anterior to the top in I and J. Scales, 1 mm in A, B and F; 250 $\mu$m in C, D and G; 400 $\mu$m in E; 500 $\mu$m in H, I and J.

element directs the expression of a transposase/$\beta$-gal fusion protein that is localized to the nucleus. When *lac-Z* genes are fused to the *hsp70* gene (HZ element) and the *ftz* gene (FZ element), $\beta$-gal produced from these elements is localized to the cytoplasm. In our P-element, P-lwB, *lac-Z* expression is controlled by the weak constitutive P transposase promotor as in the PZ element, so it is reasonable to expect $\beta$-gal to be nuclear, not cytoplasmic. To clarify this, we compared $\beta$-gal staining in P-inserted strains with Feulgen staining in *Canton-S* which reveals cell nuclei (Fig. 2C and D). With $\beta$-gal staining (Fig. 2C), stained nuclei were distinctly recognizable from the cytoplasms in organs, including exocrine glands and the digestive duct. Such a characteristic staining pattern was found also in the same organs by Feulgen staining (Fig. 2D). These organs consist of large cells in which the cytoplasm occupies most of the space. Other organs including the CNS and imaginal discs, however, were rather

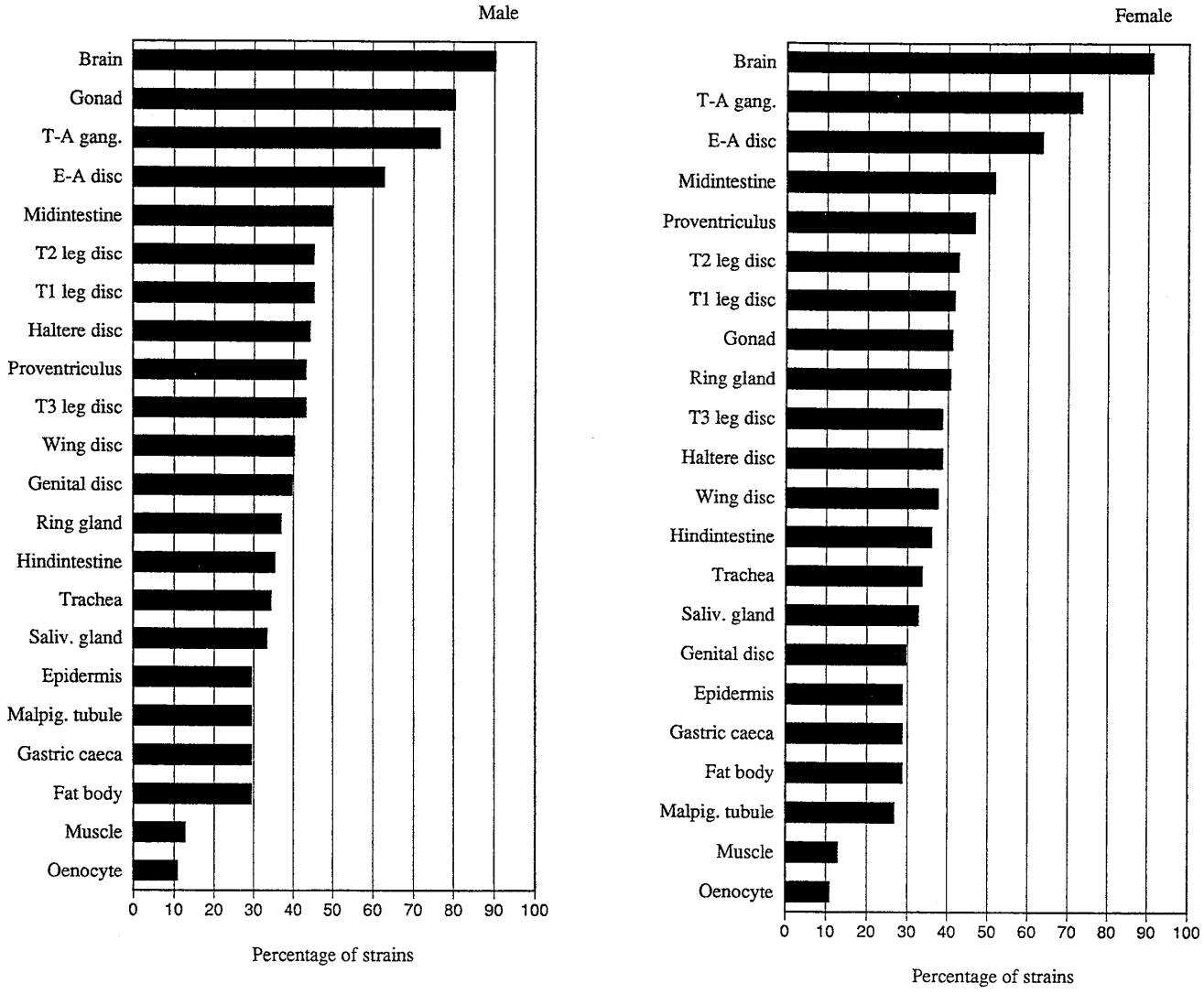



**Fig. 3** The frequency of gene expression in 22 organs and tissues in the male (left) and female (right)
Each bar represents the percentage of *lac-Z* expression of an organ among 101 strains examined. The organs are arranged from higher to lower frequencies. *lac-Z* Expression was analyzed in 101 strains randomly sampled from fertile strains, separately in the male and female.
Abbreviations are the same as in Fig. 1.

homogeneously stained whenever *lac-Z* was expressed (Fig. 2C). The same staining pattern was observed again in the Feulgen staining (Fig. 2D). This staining pattern could mean that the homogeneously stained organs consist of small packed cells in which the nucleus occupies most of the space. These results confirmed that the $\beta$-gal produced from the P-lwB element is nuclear as described in Mlodzik and Hiromi (1992)[16].

**Frequency of *lac-Z* expression in individual organs and tissues**

The frequency of *lac-Z* expression for individual organs was similar between males and females (Fig. 3), except in the gonads and genital discs. Thus, the frequency for each organ, except the genital discs and gonads, is described as an average of male and female frequencies.

The histochemical detection showed diversity in the presence or absence of *lac-Z* expression among different organs and strains. Frequency of *lac-Z* expression for a certain organ within the 101 strains was determined in 22 different organs and tissues (Fig. 3): the average frequency for 22 organs and tissues was 41.3%. The frequency, however, differed among organs. The organs can be divided into three groups according to frequency: high, middle and low frequencies. The organs which showed higher frequencies of *lac-Z* expression were the CNS (91% in the brain, 75% in the thoracico-abdominal ganglia (T-A gang.)), and the eye-antennal discs (E-A disc, 65%). The middle group included the imaginal discs (approximately 40%, except E-A disc), tracheae (34%) and salivary glands (33%). The group that showed lower frequencies included muscles (13%) and oenocytes (11%). Males in three strains and females in four strains did not express *lac-Z* except the endogenous expression. Figure 2E～J shows examples of the *lac-Z* expression limited to certain organs and tissues observed in several different strains. Some strains expressed $\beta$-gal both in the CNS and the imaginal discs but not in other organs (Fig. 2E), while others expressed in the brain and intestine (Fig. 2F), in a specific area of epidermis (Fig. 2H), or in the gonads (Fig. 2I and J). In some strains a characteristic pattern was found within the wing discs (Fig. 2E), and within the brain (Fig. 2F).

The frequency of *lac-Z* expression in the CNS was found as high as 94%. High frequency expression in the CNS has been reported[16)~18)]. In those strains showing *lac-Z* expression in the CNS, other organs and tissues usually also showed *lac-Z* expression. Five strains showed expression restricted to the CNS alone: two strains in the male and four strains in the female (one strain expressed *lac-Z* both in the male and female) showed the restricted staining in entire CNS (both in the brain and T-A gang). Four strains showed expression restricted to the brain: one in the male and four in the female (one strain is common to them). There were several different patterns of expression in the CNS. Some strains showed expression in a characteristically localized area: in the optic lobes of the brain, in the lateral sides of the T-A gang.; some strains showed it in a certain group of neurons, for example, mushroom body neurons; some strains in homologous neurons in each thoracico-abdominal neuromere; and some strains in several bilateral neurons in the brain (data not shown).

The highest percentage of *lac-Z* expressions was obtained for the eye-antennal discs (65%) among imaginal discs. Expression in the other imaginal discs, such as the haltere discs and three leg discs, was about 40%.

In the gonad, the frequency of *lac-Z* expression showed a difference between the male and female: 80% in the male vs. 41% in the female. Such a sexual difference was also found in the genital discs: 40% in the male vs. 30% in the female. Figure 2I and J shows male gonads (testes; 2J) and female gonads (ovaries; 2I) both expressing *lac-Z* gene. The pattern of *lac-Z* expression in the gonads varies among strains. Some showed uniform expression, while others showed localized expression: in the anterior or

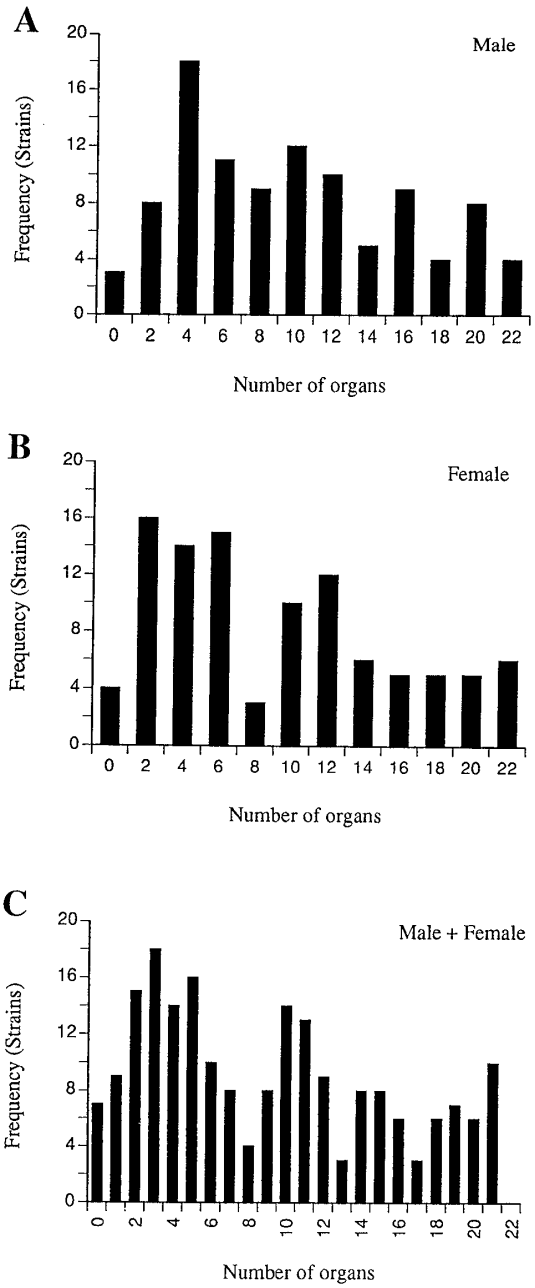



**Fig. 4** Simultaneous gene expression in different organs

The figure on the abscissa indicates the number of organs which showed *lac-Z* expression simultaneously in each strain. In A (male) and B (female), all even numbers of the abscissa represent that number (n) and that number less one (n-1). Frequency, on the ordinate, was for numbers of strains which shared the same number of *lac-Z* expressed organs, divided by the total number of strains (101). The average number of *lac-Z* expressed organs among 101 strains was 4.4 both in the male and female. In C, the frequencies of strains, for both the male and female, were simply summed (male + female). Abbreviations are the same as in Fig. 1.

posterior halves, partially anterior or posterior. Such a variety in expression was also found in the genital discs (data not shown).

Some areas of anterior and posterior portions of the midintestine were strongly and uniformly stained where innate *lac-Z* was expressed. In other parts of the digestive system, such as salivary gland, proventriculus, other areas of midintestine, gastric caeca, hindintestine and Malpighian tubules, *lac-Z* expression was found distinctly in the nuclei as mentioned above. The percentage of strains which showed *lac-Z* expression in the whole digestive duct (the proventriculus and intestine) was 16.1%, but for the majority of strains, only some part(s) expressed *lac-Z*. The frequency of *lac-Z* expression was 33% in the salivary gland, 45% in the proventriculus, 51% in the midintestine, 36% in the hindintestine, 29% in the gastric caeca, and 28% in the Malpighian tubules. The *lac-Z* expression in digestive duct showed highly diverse patterns. Moreover, *lac-Z* expression revealed many distinct transverse and longitudinal boundaries along the digestive duct, as reported[16].

Referring to the epidermis, the percentage of *lac-Z* expression was low (29%). Expression was found in all epidermal cells of thoracico-abdominal segments (Fig. 2G) in all epidermal-staining strains except a few strains: it was found in row(s) of epidermal cells in every segment in six strains and in the terminal abdominal segment (A8) in one strain (Fig. 2H).

In muscles and fat bodies, the percentage of *lac-Z* expression was lowest, 13% and 29% respectively, and was found in the whole body, and not in part.

**Simultaneous gene expression in different organs**

As mentioned above, in many strains the *lac-Z* gene was expressed in several organs and tissues simultaneously. Therefore, we examined the number of organs that showed *lac-Z* expression simultaneously in each strain. Then we summed up the number of strains which had the same number of *lac-Z* expressed

organs (Fig. 4). The distribution seems rather flat and spreads over a wide range, from the groups expressing in only a few organs to the ones expressing in almost all the organs examined. The peak of frequency of gene expression was between two to five organs, and the average number of expressed organs was 4.4 both in the male and female. Of the 101 strains examined, male in three strains and female in six strains showed expression in only one organ. These strains expressed the *lac-Z* gene in the brain for four strains (three strains only in the female and one strain both in the male and female), in the midintestine for two strains (one in the male and one in the female), in the gonad for one strain (male), and in the proventriculus for one strain (female).

**Gene expression correlation between two organs**

It would be of interest knowing whether there are any relationships in *lac-Z* expression between organs or tissues, thus we analyzed the organ to organ correlation in the male and female respectively. If expression occurs by chance between organ A and organ B, the probability of a simultaneous expression is the product of the expression percentages of organ A and B: however, it would differ from the simple product if any specific correlation existed between organ A and B.

To compare values obtained from the experiment with those from calculation, two columns were displayed side by side over 21 combinations. For example, combinations between the brain and 21 other organs are shown for the male (left) and the female (right) in Fig. 5A. There was an overall tendency for experimental values to be higher than derived from calculation. Among 231 possible combinations between the 22 organs, 228 combinations showed higher experimental values in the male and 229 combinations in the female; conversely, only three combinations showed lower experimental values in the male and two combinations in the female. Among the higher combinations, the combinations showing exceptionally higher experimental values than calculated ones were found in combinations with the imaginal discs and with organs of digestive system. This is a plausible result implying that the same genes may be involved in organs within a group. No combinations revealed large sexual differences except combinations with the gonad. Figure 5B shows the combinations in the gonad with 21 other organs. In the female, the combinations of the gonad with imaginal discs showed much higher experimental values than calculated values, as opposed to the male.

## Discussion

In this study, we used P-element inserted strains with a *lac-Z* fusion gene, and analyzed spatial patterns of *lac-Z* expression in 101 strains randomly sampled from the fertile strains. Twenty-two major organs and tissues, including CNS, imaginal discs and digestive system, were examined in third-instar larvae. The P-insertions were previously thought to have been randomly scatterd throughout the genome[19]. Engels[20] suggested, however, that P-element insertion sites are not randomly distributed within the genome; saturation for domains of gene expression was not possible using P-element insertions, indicating that there may have been genes resistant to insertion which were not detected. P-elements are also known to insert with disproportionate frequency into certain sites, and preference for which may vary with transposon structure itself[21]. The data in the present study should therefore be interpreted considering the above limitation.

Expression of the *lac-Z* gene showed diverse patterns among different organs and tissues, and moreover, among different strains. The frequency of *lac-Z* expression was extremely high, and almost all strains showed some *lac-Z* expression to a greater or lesser degree. Among the organs and tissues examined, the CNS showed the highest *lac-Z* expression (91% in the brain, 75% in the thoracico-abdominal ganglia), followed by

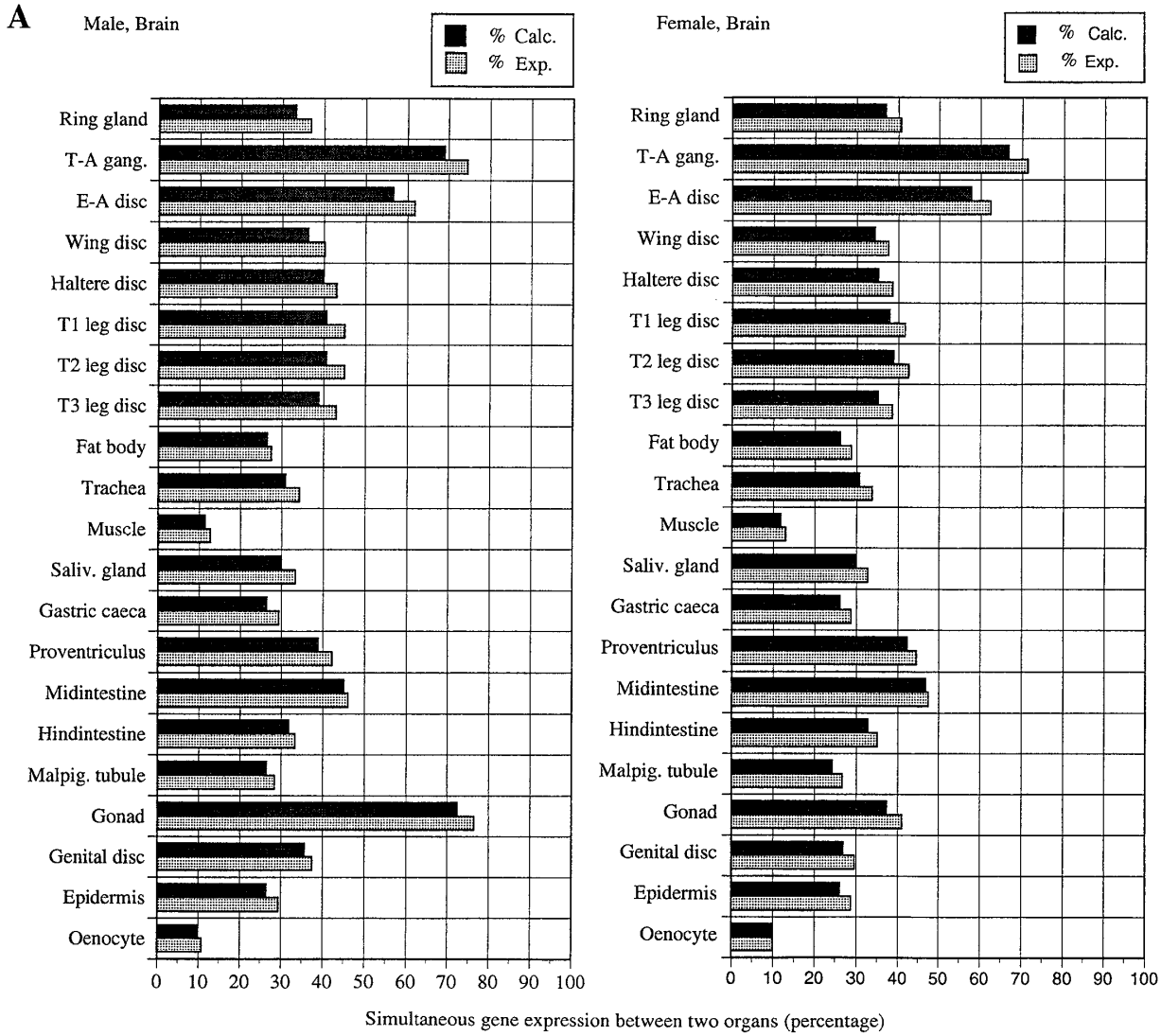



**Fig. 5** Relationships of gene expression between two different organs

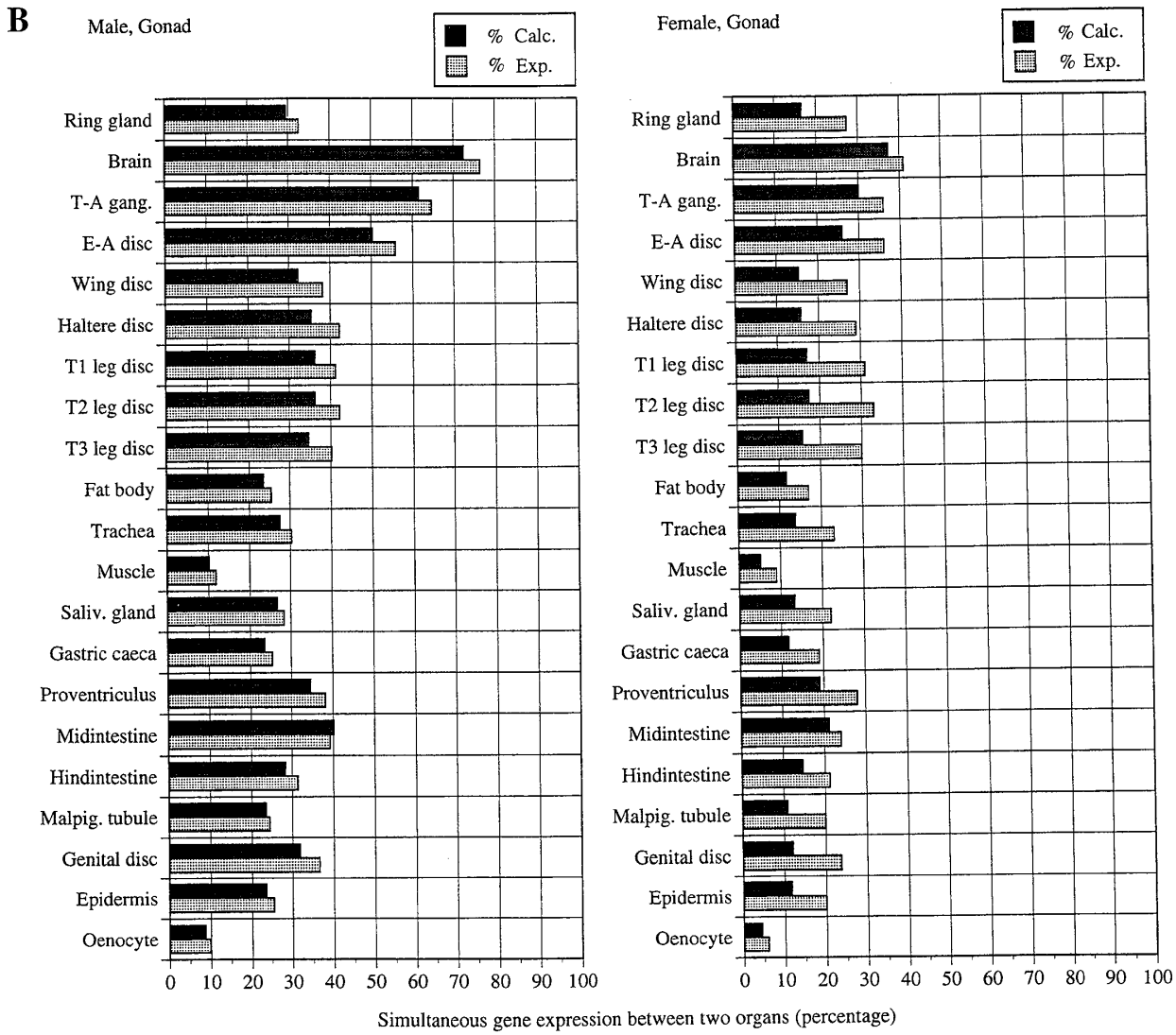
Simultaneous *lac-Z* gene expression between two different organs was counted and then divided by the total number of strains (101), resulting in a percentage of simultaneous gene expression of the experiment (experimental value: % Exp., gray bars). Corresponding calculated value (% Calc., black bars) was the simple product of the percentage expression of individual organs. One organ was combined with 21 other organs, resulting in 231 total combinations among organs, both in the male and female. Abbreviations are the same as in Fig. 1.

**A**: An example of percentage of simultaneous gene expression for combinations of the brain with 21 other organs, both in the male (left) and female (right). Note that the experimental values are higher than the calculated values. Higher experimental values were obtained in most combinations (228 in the male and 229 in the female among the 231 total combinations).

**B**: Percentage of simultaneous gene expression showing sexual differences. The combinations of the gonad with imaginal discs in the female (right), shows much higher experimental values than calculated ones, as opposed to the male (left).

imaginal discs (30% to 65%) and the digestive duct (30% to 50%), while, muscle and oenocyte showed the lowest frequencies of *lac-Z* expression, 13% and 11% respectively. Similar results have been reported by other investigators[17)22)].

Simultaneous gene expression in different organs showed the distribution in a wide range, from the groups of expressing in only one or a




few organs to the ones in almost all the organs. Some strains showed the *lac-Z* expression limited to the small number of cells, and specific to a limited region of tissues. Such a type of expression observed in organs or tissues like CNS, imaginal discs and gut tube. The CNS, which is composed of many different cell types, showed a high frequency of expression and was expressed differently from strain to strain. Bier et al.[22] pointed out that organs with frequent gene expression, such as the nervous system, may reflect a much more complex organ consisting of many different cell types. Another possible explanation is that high frequency gene expression in certain organs may reflect a dynamic developmental process, especially important in the morphogenesis of imaginal discs as well as the CNS reorganization. The wandering stage of third-instar larvae was used to reveal *lac-Z* expression in this study. From the late third-instar larva to pupa, ecdysteroid titers fluctuate dynamically[23]. During the third-instar, two small ecdysteroid fluctuations occur, the later of which is presumably responsible for initiating wandering behavior and protein synthesis. Following the wandering stage, there is a large peak in ecdysteroids which is responsible for the formation of puparium (pupariation), a process requiring a large amount of protein synthesis. Taken as a whole, *lac-Z* gene expression during the wandering stage may reflect the expression of a large number of genes, and sequential and/or parallel events of protein synthesis.

Another important finding was the difference between the experimental value of simultaneous gene expression frequency between two organs and the calculated value obtained by simple multiplication of individual gene expression frequencies. Almost always, the experimental values were found to be higher than the calculated ones in possible combinations between 22 organs: only two (in the female) to three (in the male) combinations showed lower experimental values. It is possible that much higher experimental values reflect the presence of genes necessary for basic cellular processes, such as housekeeping genes, or alternatively, it may reflect many organs sharing the cellular processes in common, if such genes do not have a housekeeping function. The morphogenesis during the wandering stage may also be involved in simultaneous gene expression. For example, ecdysteroids may trigger gene expression giving rise to many events of protein synthesis. Many genes expressed simultaneously in many different organs and the average number of organs showing simultaneous gene expression was 4.4 both in the male and female, supporting the above possibilities. Other possibilities still remain, for example, *lac-Z* expression may not reflect the true expression pattern of some genes, and the frequent simultaneous expression in many organs may be a result of incidental accompaniment by neighboring genes.

Our data also indicates some characteristic differences between males and females. The frequency of *lac-Z* expression in male gonad (80%) was about double that in the female gonad (40%), which may be related to the maturation level and consequently, gonad size. Typically, the testis is more than ten times larger than the ovary at the wandering stage, and is considered to be at a much more mature level than the ovary. This is probably related to the developmental process in which the testis grows and starts to differentiate at the larval stages, while the ovary starts to differentiate following the pupal stages[24].

**Acknowledgment**

We are grateful to Prof. Yoko Hashimoto, Tokyo Women's Medical College, for her kind support and encouragement. We thank Dr. Daisuke Yamamoto, Mitsubishi-Kasei Institute of Life Sciences, and Dr. Gerhard M. Technau, Universität Mainz, for the generous gift of the fly strains. We would also like to thank Mr. Akihito Ishihara and Miss Tomoyo Shibazaki for keeping the fly strains and for their technical assistance, and Mr. Phillip Chilton and Miss Andrea Chan for proof reading the manuscipt.

This research was partly supported by a special coordination fund for Promoting Science and Technology (SCF) in the Basic Research Core System by the Science and Technology Agency (STA), and Grants-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, Sports and Culture of Japan to A.K.

**References**

1) **Rubin GM, Spradling AC:** Genetic transformation of *Drosophila* with transposable element vectors. Science **218**: 348-353, 1982
2) **Spradling AC, Rubin GM:** Transposition of cloned P-elements into *Drosophila* germ line chromosomes. Science **218**: 341-347, 1982
3) **O'Kane C, Gehring WJ:** Detection *in situ* of genomic regulatory elements in *Drosophila*. Proc Natl Acad Sci USA **84**: 9123-9127, 1987
4) **Fasano L, Kerridge S:** Monitoring positional information during oogenesis in adult *Drosophila*. Development **104**: 245-253, 1988
5) **Yokokura T, Ueda R, Yamamoto D:** Phenotypic and molecular characterization of *croaker*, a new mating behavior mutant of *Drosophila malanogaster*. Jpn J Genet **70**: 103-117, 1995
6) **Cooley L, Kelley A, Spradling A:** Insertional mutagenesis of the *Drosophila* genome with single P elements. Science **239**: 1121-1128, 1988
7) **Bellen HJ, O'Kane CJ, Wilson C et al:** P-element-mediated enhancer detection: a versatile method to study development in *Drosophila*. Genes Dev **3**: 1288-1300, 1989
8) **Wilson C, Person RK, Bellen HJ:** P-element-mediated enhancer detection: an efficient method for isolating and characterizing developmentally regulated genes in Drosophila. Genes Dev **3**: 1301-1313, 1989
9) **Kipple DC, Macintyre RJ:** Cytogenetic mapping and isolation of mutations of the $\beta$-gal locus of

*Drosophila melanogaster*. Mol Gen Genet **198**: 75-83, 1984

10) **Prokop A, Technau GM:** The origin of postembryonic neuroblasts in the ventral nerve cord of *Drosophila melanogaster*. Development **111**: 79-88, 1991
11) **Jan LY, Jan YN:** Properties of the larval neuromuscular junction in *Drosophila melanogaster*. J Physiol **262**: 189-214, 1976
12) **Karr TL, Alberts BM:** Organization of the cytoskeleton in early *Drosophila* embryos. J Cell Biol **102**: 1494-1509, 1986
13) **Simon JA, Sutton CA, Lobell RB et al:** Determinants of heat shock-induced chromosome puffing. Cell **40**: 805-817, 1985
14) **Feulgen R, Rossenbeck H:** Mikroskopisch-chemischen Nachweis einer Nucleinsaure von Typus der Thymonucleinsaure und die darauf beruhende elektive Farbung von Zellkernen in mikroskopischen Praparaten. Hoppe-Seyler Z Physiol Chem **135**: 203-248, 1924
15) **Murakami R, Shigenaga A, Kawano E et al:** Novel tissue units of regional differentiation in the gut epithelium of *Drosophila*, as revealed by P-element-mediated detection of enhancer. Roux's Arch Dev Biol **203**: 243-249, 1994
16) **Mlodzik M, Hiromi Y:** Enhancer trap method in *Drosophila*: its application to neuronbiology. *In* Methods in Neurosciences, Vol. 9 pp 397-414, Academic Press, New York (1992)
17) **Datta S, Stark K, Kankel DR:** Enhancer detector analysis of the extent of genomic involvement in *Drosophila melanogaster*. J Neurobiol **24**: 824-841, 1993
18) **So S. Tsujimura H:** Characterization of P element enhancer trap vector HZ in 3rd instar larvae in *Drosophila melanogaster*. Bull Fac Gen Ed Tokyo Univ Agri Tech **31**: 201-211, 1994 (in Japanese)
19) **Spradling A:** P-element-mediated transformation. *In Drosophila*, A Practical Approach (Roberts DB ed) pp 175-197, IRL Press, Oxford (1986)
20) **Engles WR:** P-elements in *Drosophila*. *In* Mobile DNA (Berg DE and Howe MM eds) pp 437-484, American Society of Microbiology, Washington, D. C. (1989)
21) **Berg CA, Spradling AC:** Studies on the rate and site-specificity of P-element transposition. Genetics **127**: 515-524, 1991
22) **Bier E, Vasessin H, Shepherd S et al:** Searching for pattern and mutation in the *Drosophila* genome with a P-*lac Z* vector. Genes Dev 3: 1273-1287, 1989
23) **Riddiford LM:** Hormones and *Drosophila* development. *In* The Development of *Drosophila melanogaster* (Bate M and Arias AM eds) pp 899-939, Cold Spring Harbor Laboratory Press, New York (1993)
24) **Bownes M, Dale L:** Chapter 2, Gametogenesis. *In* A Handbook of *Drosophila* Development (Ranson R ed) pp 31-66, Elsevier Biomedical Press, Amsterdam (1982)
25) **Bodenstein D:** The postembryonic development of *Drosophila*. *In* Biology of *Drosophila* (Demerec M ed) pp 275-367, Cold Spring Harbor Laboratory Press, New York (1994)

# エンハンサートラップ法を用いた *Drosophila melanogaster* における遺伝子発現パターンの解析

東京女子医科大学　第一生理学教室（主任:　橋本葉子教授）
町山（マチヤマ）　悦子（エツコ）・小松（コマツ）　明（アキラ）

キイロショウジョウバエ *Drosophila melanogaster* のゲノムに，jump-start 法を用いて，レポーター遺伝子として大腸菌の β-galactosidase 遺伝子（*lac-Z*）を組み込んだ P 因子（P-lwB）挿入系統で，エンハンサーによる遺伝子発現を観察した．101の妊性系統を用い，3 令幼虫の主要な22の器官と組織における遺伝子発現を，β-galactosidase の活性を組織化学的に検出して（X-gal 法）解析した．*lac-Z* の発現は器官や組織によって，また，系統によって多様な発現パターンを示した．*lac-Z* の発現頻度は，中枢神経系で最も高く（脳で91%，胸腹部神経節で75%），成虫原基（30～65%）や消化管（30～50%）は中程度で，筋肉（13%）とエノサイト（11%）では低値を示した．中枢神経系での発現率が高いのは，中枢神経系を構成する細胞の種類が多いことによるものと考えられる．また，3 令幼虫の後期はホルモン分泌や蛋白合成の盛んな発生過程にあり，中枢神経系とともに成虫原基での発現率が高いのは，これらの器官でダイナミックな組織再編成が生じていることを反映しているものと考えられる．複数器官での同時発現率を調べると，少数の器官で特異的に発現するものから多数同時発現を示すものと多様であり，その平均値は雌雄とも4.4であった．また，2 つの器官で同時に発現する頻度は，両者の発現率の積よりも，ほとんど全部の組み合わせで高い値を示した．このことは，多くの器官に共通する細胞過程に必要な遺伝子，あるいは，ハウスキーピング遺伝子のような細胞の基本過程に共通して必要な遺伝子の存在を示唆する．