**Genetic analysis of variation in the number of
primary spermatocytes per cyst in
*Drosophila virilis***

Yuzuru OGUMA and Haruo KUROKAWA

*Institute of Biological Sciences, University of Tsukuba,
Tukuba, Ibaraki 305*

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ABSTRACT

Two experiments were performed with the aim of clarifying the genetic basis of variation in the number of primary spermatocytes per cyst in the B12 strain of *Drosophila virilis* and identifying the chromosome responsible for the variation. First, crosses between B12, showing a mean cell number of 11.81, and strain TK with a mean of 7.91, were performed. From the results obtained, it was considered that there were two factors, i.e., a recessive gene and certain modifiers, which shifted the cell number toward a higher value in B12. Second, chromosomal analysis with a marker strain and the B12 strain revealed that the third chromosome of B12 was responsible for the major effect of changing the primary spermatocyte number, although this chromosome did not exert a sufficient effect alone, and furthermore that at least one of the modifiers located on the second chromosome.

1. INTRODUCTION

Spermatogonial proliferation in *Drosophila* involves a special series of cell divisions in the testis. Each definitive spermatogonium generally exhibits a characteristic number of repeated mitotic divisions during the multiplication period and gives rise to a constant number of primary spermatocytes per cyst (Tihen, 1947; Cooper, 1950; Hannah-Alava, 1965; Meyer, 1968; Courot et al., 1970; Kurokawa and Hihara, 1976; Lindsley and Tokuyasu, 1980; Oguma et al., 1987). According to the cell numbers present in a cyst, Kurokawa and Hihara (1976) classified 78 drosophilid species into 4 regular classes, $8(2^3)$, $16(2^4)$, $32(2^5)$ and $64(2^6)$ cells, and one exceptional species. In their study, *D. virilis* was tentatively assigned to the 8-cell class, because its number of mitotic divisions was regarded as 3. They also drew attention to the occasional appearance of cysts containing 7 cells along with those containing the ordinary number of 8. Liebrich et al. (1982) and Oguma et al. (1984) reported that the cell number in *D. virilis* showed a further variation when greater numbers of cysts were examined. It was also clarified by our study that this variation in cell number was rather constant both within and between strains of this species. Among the various strains examined, *D. virilis* B12 showed an extremely high cell number, although

male fertility in this strain was significantly low, the males being able to produce only one tenth of the total progeny that was usual for strain TK, having the ordinary number of 8 cells (Oguma et al, 1984).

The present study was carried out in order to elucidate the genetic basis for the variation of cell number in *D. virilis* and to identify the chromosome responsible.

2. MATERIALS AND METHODS

Crosses between TK and B12 strains

Strain TK used in this study was derived from a single female fly caught in Tokyo and had been maintained as a stock for about 45 years in the laboratory. Strain B12 was derived from a single female fly caught in Ehime Prefecture in 1976. The mean numbers of primary spermatocytes per cyst in these two strains were significantly different from each other (Oguma et al., 1984). The F₁ hybrids arising from reciprocal crosses between these two strains were backcrossed to B12. Observation of primary spermatocytes was performed with a technique reported previously (Oguma et al., 1984), and the number of cells present per cyst was examined using 20 cysts from each male fly.

Chromosomal analysis with a marker strain

Analysis with a marker strain and the B12 strain was carried out in order to reveal the particular chromosome responsible for increasing the primary spermatocyte cell number in B12. The marker strain had five visible mutant charac-

Fig. 1. Mating scheme for chromosomal analysis using the marker and B12 strains. Mutant names of gene symbols and their chromosomal numbers are as follows: b (broken, II), gp (gapped, III), cd (cardinal, IV), pe (peach, V) and gl (glossy, VI).

teristics: broken (b, II), gapped (gp, III), cardinal (cd, IV), peach (pe, V) and glossy (gl, VI). As shown in Fig. 1, 5 males of F_1 hybrid were crossed with 5 females of marker-strain, and male offspring of five genotypes at the X_1 generation from that cross (each abbreviated to II-VI) were individually crossed with 5 females of B12. The numbers of primary spermatocytes per cyst per male were scored at the X_2 generation. The B12 strain, marker strain and their hybrid F_1 males were also scored as a control. If the factor(s) increasing the cyst number located on a particular chromosome in B12, flies homozygous for that chromosome would show a high number of primary spermatocytes per cyst, and also the ratio between males showing the standard number and those showing the higher one would be 1:1.

3. RESULTS

Crosses between TK and B12 strains

Figs. 2a, b and c show the frequency distributions of the numbers of primary spermatocytes per cyst of strains TK and B12, and of their F_1 hybrids, respectively. TK with a mean cell number of 7.91 ± 0.01 , being very close to 8, was considered to be of the ordinary type, in which 3 multiplication divisions were assumed to have occurred. Strain B12, on the other hand, showed an extremely high mean cell number of 11.81 ± 0.05 . The mean cell numbers of the F_1 hybrid males obtained reciprocally from the parental crosses were 8.51 ± 0.01 and 8.72 ± 0.02 , respectively (Figs. 2b and c). These values, showing no significant

Fig. 2. Frequency distributions of the numbers of primary spermatocytes per cyst per male.

a, The parental strains. TK, $N=19$; B12, $N=21$.

b, F_1 hybrids from TK females and B12 males. $N=20$.

c, F_1 hybrids from B12 females and TK males. $N=22$. Arrows show the means for each cross.

difference between them ($P > 0.05$, Mann-Whitney U-test), were both closer to the mean for TK than to the mid-parent value (9.86) but were significantly different from TK ($0.01 < P < 0.05$ for both pairwise combinations, Mann-Whitney U-test). This lack of difference between the two F_1 values suggested that neither the X nor the Y chromosome had any significant effect in increasing the cell number and also showed no maternal effect. Fig. 3 shows the frequency distributions of the cell numbers in the backcross progeny (BC_1). The mean values found in the BC_1 males were clearly higher than those found in the F_1 males, and exhibited a much wider variation. There were no significant differences between any combination among the four mean values in BC_1 males ($P > 0.05$ for all pairwise combinations, Mann-Whitney U-test).

Fig. 3. Frequency distributions of the numbers of primary spermatocytes per cyst per male from backcrosses to B12. a, $N=99$; b, $N=64$; c, $N=58$; d, $N=70$. Arrows show the means for each cross.

Chromosomal analysis

Table 1 shows the results of chromosomal analysis. Abbreviations (II-VI) are the same as those in Fig. 1. Males observed at the X_2 generation had the same chromosomal constitution, being heterozygous with B12 and the marker strain without any particular chromosome to test. Since the males for which the chromosome classes were tested except for the second and the third chromosome

Table 1. Number of males with regard to number of primary spermatocytes per cyst per male at the X₂ generation

Strain or tested chromosome	Number of observed males	Number of primary spermatocytes/cyst/male					Mean $\pm$ S.E.M.
		8.0 -8.9	9.0 -9.9	10.0 -10.9	11.0 -11.9	12.0 -12.9	
Control							
B12	50				31	19	11.85 $\pm$ 0.06
Marker	50	38	12				8.87 $\pm$ 0.03
F ₁ Hybrid	50	15	35				9.14 $\pm$ 0.04
Results at X ₂ (Experiment at X ₁)							
II*	110 (11)**	9	100	1			9.31 $\pm$ 0.03
III	110 (11)	14	44	43	9		9.83 $\pm$ 0.11
IV	100 (10)	18	82				9.19 $\pm$ 0.02
V	100 (10)	46	54				8.91 $\pm$ 0.03
VI	80 (8)	22	58				9.11 $\pm$ 0.02

* II-VI indicate the chromosomal number tested. See Fig. 1.

** The number in parentheses indicates total number of males of the X₁ generation mated to test for each chromosome class.

class (II and III) were all within the range of 8.0–9.9 primary spermatocyte number of the marker strain, we considered that these chromosomes had no major effect on increasing the cell number. The third chromosome class (III) showed a feature that was different from the others, in addition to the standard range (8.0–9.9, total 58 males), the higher one (10.0–11.9, total 52 males) was segregated, the ratio between them being 1:1 ($0.3 < P < 0.5$, χ^2 -test). We considered that 58 males having 8.0–9.9 primary spermatocytes per cyst were heterozygous for the marker strain and the third chromosome of B12, whereas 52 males with 10.0–11.9 spermatocytes were homozygous for B12, although the latter did not completely agree with that shown by the B12 strain.

The second chromosome (II) showed the significantly higher value of 9.31 ± 0.03 primary spermatocytes number per male than that of 9.14 ± 0.04 in F₁ hybrid ($0.01 < P < 0.05$, Mann-Whitney U-test), but there were no significant difference between F₁ hybrid and any chromosome of the IV, V and VI, respectively ($P > 0.05$ for all pairwise combinations, Mann-Whitney U-test) (Table 1).

4. DISCUSSION

It has been assumed that a certain genetic system plays a role in determining a constant number of mitotic divisions during the period of cell multiplication in the testis, which leads to the production of a constant number of primary spermatocytes. Although the number of mitotic divisions seems to be generally species-specific (Kurokawa and Hihara, 1976), a wide variation in the number of primary

spermatocytes has been reported in *D. virilis* by Liebrich et al. (1982) and Oguma et al. (1984). On the other hand, variations in temperature and nutritional conditions during fly-rearing do not influence the mean number of primary spermatocytes, supporting the notion of a genetically determined cell number per cyst (our unpublished data).

For purposes of examining the genetic basis of spermatocyte number, B12 has an advantage in that its mean cell number of 11.81 is markedly higher than that of the ordinary number of 8 in *D. virilis*. As shown in Figs. 1b and c, the mean cell numbers of F_1 males derived from reciprocal crosses between TK and B12 were very close to each other. The values of 8.51 and 8.72 were much closer to that of TK (7.91) than to that of B12 (11.81). Thus it appears that there is a major, recessive factor which exert a marked influence on the mitotic cell divisions occurring at the multiplication stage in the B12 strain, and that certain modifiers shift the mean toward a higher value in B12. Comparing the data for parent flies with those of the F_1 hybrids, the frequency distribution pattern with regard to the cell number does not seem to be affected by any sex-linked factor, but rather by an autosomal one. The lack of bimodality in the distribution of the cell numbers in different backcross progeny shows that the genetic pattern of the cell number cannot be explained only in terms of a major factor alone (Fig. 3).

This possibility is supported by the results of chromosomal analysis, which showed that the third chromosome is responsible for increasing the number of primary spermatocytes per cyst in B12, but not sufficiently toward the higher value. However some additional modifiers were also found to be located on the second chromosome (II) (Table 1).

The present chromosomal analysis did not necessarily reveal the factor to be a single one. Upon comparison of the frequency distributions of the numbers of primary spermatocytes per cyst per male in the BC_1 generation produced by F_1 hybrid females with those produced by males, no differences were found. If the factor on the third chromosome were not single, hybrid females could have many kinds of recombinants. Therefore a difference would be expected in the frequency distribution patterns of BC_1 males whether they were derived from hybrid males or hybrid females (Fig. 3a, c and 3b, d). We conclude that the present recessive factor on the third chromosome responsible for increasing the cell number is single allele.

A phenomenon similar to the significant difference between F_1 hybrids from the TK $\times$ B12 cross and the TK strain (Fig. 1, mean in TK=7.91, F_1 hybrid=8.51, $0.01 < P < 0.05$, Mann-Whitney U-Test) was also observed for the difference between F_1 hybrids from the marker $\times$ B12 cross and the marker strain (Table 1, marker strain=8.87 $\pm$ 0.03, F_1 hybrid=9.14 $\pm$ 0.04, $0.01 < P < 0.05$, Mann-Whitney U-Test). We, therefore, consider that TK and the marker strain show incomplete dominance against the present recessive factor.

Lifschytz and Hareven (1977) argued that spermatid morphogenesis was totally

dependent on the developmental processes occurring at the primary spermatocyte stage. In the present study, the primary spermatocytes of strain B12 appeared to be morphologically normal upon light microscopic observation. During spermiogenesis, however, many spermatids exhibited degenerated or transformed mitochondrial derivatives and axoneme as revealed by electron microscopy (our unpublished data). These ultrastructural abnormalities might produce some functional derangement within the spermatozoa, leading to a subsequent reduction of male fertility in B12 (Oguma et al., 1984).

Finally, the recessive factor and modifiers affecting the mitotic division of definitive spermatogonia, which we first discovered in the present paper, might be related to asynchronous mitotic cell division, as has been suggested by Hanna et al. (1982) and Lieblich et al. (1982).

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REFERENCES

- COOPER, K. W. (1950) Normal spermatogenesis in *Drosophila*. In: *Biology of Drosophila*, (ed.: M. Demerec), pp. 1–61. Hafner Publ. Co., New York.
- COUROT, M., HOCHEREAU-DE REVIERS, M. T. and ORTAVANT, R. (1970) Spermatogenesis. In: *The Testes*, (eds.: A. D. Johnson, W. R. Gones and N. L. Vandemark), Vol. I, pp. 339–432. Academic Press, New York.
- HANNA, P. J., LIEBRICH, W. and HESS, O. (1982) Evidence against a (2)ⁿ synchronous increase of spermatogonia to produce spermatocytes in *Drosophila hydei*. *Gamete Research* **6**, 365–370.
- HANNAH-ALAVA, A. (1965) The premeiotic stage of spermatogenesis. *Adv. Genet.* **13**, 157–226.
- KUROKAWA, H. and HIHARA, F. (1976) Number of first spermatocytes in relation to phylogeny of *Drosophila* (Diptera; Drosophilidae). *Int. J. Insect Morphol. Embryol.* **5**, 51–63.
- LIEBRICH, W., HANNA, P. J. and HESS, O. (1982) Evidence for asynchronous mitotic divisions in secondary spermatogonia of *Drosophila*. *Int. J. Invert. Reprod.* **5**, 305–310.
- LIFSCHYTZ, E. and HAREVEN, D. (1977) Gene expression and the control of spermatid morphogenesis in *Drosophila melanogaster*. *Devel. Biol.* **58**, 276–294.
- LINDSLEY, D. L. and TOKUYASU, K. T., (1980) Spermatogenesis. In: *The Genetics and Biology of Drosophila*, (eds.: M. Ashburner and T. R. Wright), Vol. 2d, pp. 225–294. Academic Press, New York.
- MEYER, G. F. (1968) Spermiogenese in normalen und Y-defizienten Männchen von *Drosophila melanogaster* und *D. hydei*. *Z. Zellforsch.* **84**, 141–175.
- OGUMA, Y., KUROKAWA, H. and KUSAMA, T. (1987) Number of primary spermatocytes in the *Drosophila immigrans* (Sturtevant) group (Diptera: Drosophilidae). *Int. J. Insect Morphol. Embryol.* **16**, 85–89.
- OGUMA, Y., KUROKAWA, H. and SOGAWA, Y. (1984) Variation of the number of first spermatocytes in relation to fertility in *Drosophila virilis*. *Jpn. J. Genet.* **59**, 565–575.
- TIHEN, J. A. (1964) An estimate of the number of cell generations preceding sperm formation in *Drosophila melanogaster*. *Am. Nat.* **80**, 389–392.