Genet. Res., Camb. (1977), **30**, *pp.* 107–113
Printed in Great Britain

Sterile mutation in *Drosophila melanogaster**

BY TAKAO K. WATANABE AND WON HO LEE
National Institute of Genetics, Mishima 411, Japan

(*Received* 24 *January* 1977)

SUMMARY

The number of loci which are potentially able to produce sterility genes was estimated for *Drosophila melanogaster*. There appear to be, on the second chromosome, about 80 loci capable of producing male sterility and about 60 loci capable of producing female sterility. These figures seem to be considerably less than (400–500) loci responsible for lethal genes.

1. INTRODUCTION

Frequencies of recessive lethals, male-steriles and female-steriles on the second chromosomes in natural and cage populations of *Drosophila melanogaster* have been found to be roughly in a 4:2:1 ratio (Oshima & Watanabe, 1973; Watanabe, Watanabe & Oshima, 1976). These results suggest that either (1) sterile mutation rate per locus is lower than lethal mutation rate, though the number of loci capable of producing lethals and that of loci responsible for steriles are roughly equal; or (2) the number of potentially sterile loci is less than the number of lethal loci, though the mutation rate per locus is approximately the same for the two kinds of loci; or (3) selection against sterility genes, especially female-sterility genes, is stronger than against lethal genes, and therefore sterility genes are eliminated more quickly and kept at a low frequency.

Recently, chemical mutagens such as EMS have been used for induction of sterile mutations in *Drosophila*. Using this method, Gans, Audit & Masson (1975) have estimated a total of 150 sex-linked female sterile loci including the temperature-sensitive mutations. If we use this result to extrapolate to the sterile loci on the second chromosome, we should expect there to be about $150 \times 2 = 300$ loci capable of mutating to sterility since the second chromosome is about twice the length of the *X* chromosome. On the other hand, using a different method, Watanabe (1977) has estimated the number to be less than 80–100 for the second chromosome.

The present paper is an attempt to provide a better and more reliable estimate of the sterile mutation rate. The numbers of potentially sterile loci are also estimated.

2. MATERIALS AND METHODS

Two second chromosome lines were used for induction of lethal and sterility genes by EMS. One was a male-sterile line (item 7 in Table 1) which had no lethal and female-sterility genes but had a recessive male-sterility gene. Another was a female-

* Contribution no. 1111 from the National Institute of Genetics.

Table 1. *Frequencies of induced and spontaneous recessive lethals, male-steriles and female-steriles in the first, second and third chromosomes of* Drosophila melanogaster

Mutagen	Treated chromosome	No of chromosomes (N)	Frequency of lethals (l)	Frequency of ♂-steriles (d_m)	d_m/l (λ_m)	Freuqency of ♀-steriles (d_f)	d_f/l (λ_f)	Data from
1. EMS	X	4328	0·284	0·032	0·11	0·038	0·13	Kaplan *et al.* (1970)
2. EMS	X	6433	0·365	0·047	0·13	—	—	Denell, 1973
3. EMS	X	3783	0·600	0·218	0·36	—	—	Gans *et al.* 1975
4. EMS	X	2660	0·600	—	—	0·089	0·15	Gans *et al.* 1975
5. EMS	II	2493	0·698	—	—	0·161	0·23	Bakken, 1973
6. EMS	II	188	0·360	0·067	0·19	0·017	0·05	Watanabe, 1977
7. EMS	II	220	0·168	—	—	0·016	0·10	Present paper
8. EMS	II	222	0·396	0·037	0·09	—	—	Present paper
9. EMS	III	3646	0·694	—	—	0·049	0·07	Bakken, 1973
10. AF-2	II	1654	0·0085	0·0049	0·58	0·0024	0·28	Watanabe, 1977
11. Spontaneous	II	2857	0·0056	—	—	0·0004	0·07	Shapiro, 1939
12. Spontaneous	II	1612	0·0074	0·0013	0·18	—	—	Watanabe, 1977
13. Spontaneous	II	1632	0·0074	—	—	0·0019	0·26	Watanabe, 1977
14. Spontaneous	II	1180	0·0085	0·0051	0·60	0·0034	0·40	Present paper
Weighted mean (μ)			1		$0·153 \pm 0·0066$		$0·115 \pm 0·0062$	

sterile line (item 8 in Table 1) having neither lethal nor male-sterility gene, but a recessive female-sterility gene. Each sterile line had its second chromosome balanced against *Cy*, and heterozygous males (*st*/*Cy*) were treated with EMS ($2{\cdot}5 \times 10^{-3}$ M dissolved in 1% sucrose solution) for 24 h. The treated males were mated with *Cy*/*Pm* females for one day and many F_1 *Cy* males were crossed individually to *Cy*/*Pm* females. In the F_2, five pair matings of *Cy* flies were made for each treated chromosome. If non-*Cy* flies (*st*/*st*) do not appear in the following generation, the tested chromosome must carry a recessive lethal gene in addition to the original sterility gene. Among non-lethal carrying chromosomes of EMS-treated male-sterile lines, female-sterility was examined. Five non-*Cy* females (*st*/*st*) from each line were crossed to five Oregon-R males. If no larvae could be found from the cross, the tested chromosome was regarded as carrying a female-sterility gene. The test for male-sterility in the treated female-sterile lines was performed by crossing with Oregon-R virgin females in a similar way.

One hundred second chromosomes carrying no lethal and no sterility gene were isolated from a cage population. They were individually balanced with *Cy* chromosomes and each line was maintained by crossing a single heterozygous male (+ /*Cy*) to *Cy*/*Pm* females in every generation. Recessive mutations such as lethal, male-sterility and female-sterility genes were accumulated in each second chromosome for 13 generations. The spontaneous lethal or sterile mutations were detected by the method described above. The number of tests in this case was expressed as chromosome-generations, that is the product of the number of chromosomes tested in homozygous condition and the number of generations these had been allowed to accumulate mutations (item 14 in Table 1).

All experiments were carried out at 25 °C with the standard cornmeal, agar, molasses and yeast medium.

3. RESULTS AND DISCUSSION

The frequencies of induced and spontaneous sterility mutations as well as lethal mutations are shown in Table 1, together with some data from published literature. The frequency of steriles shown in the table was calculated as the frequency of steriles among non-lethal chromosomes tested (K). When the actual number of chromosomes (N) was not available from the literature, it was calculated from the lethal frequency (l) and non-lethal chromosomes (K) as follows, $N = K/(1-l)$. The mutagenic strength of the chemical treatment differed to some extent between experiments, and treated chromosomes. However, if mutations to lethality and sterility are compared within each experiment and presented as relative mutation ratios, these values are useful for estimating the standard mutation rate of sterility genes. To standardize the mutagenic effects of various mutagens on various chromosomes (involving the *X*, *II*, *III* chromosomes), the frequency of male-sterile (d_m) or female-sterile chromosomes (d_f) was divided by the frequency of lethal chromosomes (l) in each experiment. Although the ratios d_m/l ($= \gamma_m$) or d_f/l ($= \gamma_f$) are variable among experiments, they are apparently smaller than 1. That is, the sterility mutation rate per chromosome is less than the lethal mutation rate.

A statistical method of treating this kind of data is given in the Appendix by Avery Period. According to the statistics, the weighted mean ratio which is also a maximum likelihood estimate is $0{\cdot}153 \pm 0{\cdot}0066$ for d_m/l ratio and $0{\cdot}115 \pm 0{\cdot}0062$ for d_f/l ratio. If we assume the underlying assumptions imposed in the statistical theory, these values of the two ratios (d_m/l and d_f/l) appear to be significantly different, and both types of sterile mutation seem to occur at different rate. It also appears to be that values of d_m/l (or d_f/l) ratio may be significantly different among different experiments. However, before we can reach a definite conclusion, we must be certain that the distribution of the ratios is approximately normal. Although it may be revised later with more data, these weighted mean ratios are useful in an attempt to estimate the number of loci capable of producing sterile mutation.

If we suppose that sterile and lethal mutations per locus occur approximately at the same rate, and if the number (N_l) of loci capable of producing lethals is known, the number (N_s) of loci which are able to have sterile can be calculated: $N_s = N_l \times (d/l)$. Indeed Wallace (1950) and Ives (1945) estimated the number of potentially lethal loci on the second chromosome of *D. melanogaster* to be 400–500. These values have been confirmed by various other methods, and are reliable. Therefore if we assume the same rate for lethals and steriles, and if the number of lethal producing loci is 500, the number of potentially male-sterile loci is $500 \times 0{\cdot}153 = 76{\cdot}5$ and that of female-sterile loci $500 \times 0{\cdot}115 = 57{\cdot}5$.

We thank Dr Takeo Maruyama for his critical reading of the manuscript. We also thank Dr P. J. Avery for his kind derivation of the formulae in the Appendix.

REFERENCES

BAKKEN, A. H. (1973). A cytological and genetic study of oogenesis in *Drosophila melanogaster*. *Developmental Biology* **33**, 100–122.

DENELL, R. E. (1973). Use of male sterilization mutations for insect control programmes. *Nature* **242**, 274–275.

GANS, M., AUDIT, C. & MASSON, M. (1975). Isolation and characterization of sex-linked female-sterile mutants in *Drosophila melanogaster*. *Genetics* **82**, 683–704.

IVES, P. T. (1945). The genetic structure of American populations of *Drosophila melanogaster*. *Genetics* **30**, 167–196.

KAPLAN, W. D., SEECOF, R. L., TROUT, W. E. III. & PASTERNACK, M. E. (1970). Production and relative frequency of maternally influenced lethals in *Drosophila melanogaster*. *American Naturalist* **104**, 261–271.

OSHIMA, C. & WATANABE, T. K. (1973). Fertility genes in natural populations of *Drosophila melanogaster*. I. Frequency, allelism and persistence of sterility genes. *Genetics* **74**, 351–361.

SHAPIRO, N. I. (1939). The rate of spontaneous sterile mutation. *Drosophila Information Service* **11**, 50.

WALLACE, B. (1950). Allelism of second chromosome lethals in *D. melanogaster*. *Proceedings of the National Academy of Sciences, U.S.A.* **36**, 654–657.

WATANABE, T. K. (1977). Genetic variations in reproductive ability observed in natural populations of *Drosophila melanogaster*. *Advances in Invertebrate Reproduction* (ed. K. G. Adivodi and R. G. Adiyodi). *Kerala:* Peralam-Kenoth. India, **1**, 25–35.

WATANABE, T. K., WATANABE, T. & OSHIMA, C. (1976). Genetic changes in natural populations of *Drosophila melanogaster*. *Evolution* **30**, 109–118.

APPENDIX: ESTIMATION OF THE RATIO OF % STERILES TO % LETHALS

By P. J. AVERY

Institute of Animal Genetics
West Mains Road, Edinburgh, EH9 3JN, Scotland

Consider a population of chromosomes with frequency p of lethals and p_1 of steriles. A sample of N is tested and L are found to be lethal and S of the remaining $(N-L)$ are sterile. Let $l = L/N$ and $d = S/(N-L)$, then we wish to know the variance of the ratio, $\gamma = d/l$.

Then $E(l) = p$

$$E(d) = E\left(\frac{S}{N-L}\right) = E_L\left(E_{SL}\left(\frac{S}{N-L}\right)\right)$$

$$= E_L\left(\frac{(N-L)p_1}{(N-L)}\right) \text{ (as when } L \text{ is given, } S \text{ is Binomial on sample size } (N-L)\text{)}$$

$$= p_1 \,\text{prob}\,(L \neq N) \text{ (as, when } L = N,\ S = d = 0)$$

$$= p_1(1-p^N)$$

$$\simeq p_1 \text{ (as long as } N \text{ is not very small)},$$

$$\text{var}\,(l) = \frac{p(1-p)}{N} \text{(binomial sampling)},$$

$$\text{cov}\,(l,d) = E\left(\frac{SL}{N(N-L)}\right) - E(l)\,E(d)$$

$$= E_L\left(E_{SL}\left(\frac{SL}{N(N-L)}\right)\right) - pp_1(1-p^N)$$

$$= E_L\left(\frac{(N-L)p_1 L}{(N-L)N}\right) - pp_1(1-p^N)$$

$$= 0,$$

$$\text{var}\,(d) = E(d^2) - [E(d)]^2$$

$$= E_L\left[E_{SL}\left[\frac{S^2}{(N-L)^2}\right]\right] - p_1^2(1-p^N)^2$$

$$= E_L\left[\frac{(N-L)^2 p_1^2 + (N-L)p_1(1-p_1)}{(N-L)^2}\right] - p_1^2(1-p^N)^2$$

$$\simeq p_1(1-p_1)\,E\left[\frac{1}{N-L}\middle| L \neq N\right].$$

By use of summations with binomial probabilities, it can be shown that

$$E\left[\frac{1}{(N-L)}\middle| L \neq N\right] = \frac{1}{Nq} \quad (\text{where } q = 1-p).$$

Thus

$$\text{var}\,(d) = \frac{p_1(1-p_1)}{Nq}.$$

https://doi.org/10.1017/S0016672300017523 Published online by Cambridge University Press

In the above calculations, $E(x)$ is the expected value of x over all possible values of S and L, $E_{SL}(x)$ is the expected value of x over all possible values of S, given that L is known, and $E_L(x)$ is the expected value of x over all possible values of L. $E[1/(N-L)|L \neq N]$ is the expected value of $1/(N-L)$ over all values of L other than $L = N$.

Thus by using binomial distribution theory, we have found values for the mean and variances of d and l. We cannot find the mean and variance exactly for γ, but by Taylor's Series Expansion,

$$E(\gamma) = p_1/p$$

and

$$\begin{aligned} \text{var}(\gamma) &\simeq \frac{[E(d)]^2}{[E(l)^2}\left\{\frac{\text{var}(d)}{[E(d)]^2}+\frac{\text{var}(l)}{[E(l)]^2}-2\frac{\text{cov}(l,d)}{E(l)\,E(d)}\right\} \\ &\simeq \frac{p_1^2}{p^2}\left\{\frac{p_1(1-p_1)}{Nqp_1^2}+\frac{p(1-p)}{Np^2}-2\frac{0}{pp_1}\right\} \\ &= \frac{p_1^2}{Np^2}\left\{\frac{1}{q}.\frac{q_1}{p_1}+\frac{q}{p}\right\} \quad (\text{where } q_1 = 1-p_1). \end{aligned} \tag{A 1}$$

In the special case, when all N individuals can be scored both for lethality and sterility, the mathematics simplifies as there are now two independent binomial samplings of size N. Only the variance of d changes, however, and by using the same approximate Taylor's expansion formula

$$\text{var}(\gamma) = \frac{p_1^2}{Np^2}\left\{\frac{q_1}{p_1}+\frac{q}{p}\right\}. \tag{A 2}$$

For all values of the parameters (A 2) yields a smaller variance than (A 1), as you would expect, as more information is now available (i.e. number which are both lethal and sterile).

In the practical situation, we have several different readings for γ ($\gamma_1, \gamma_2, \ldots \gamma_n$, say) and we wish to know if they are significantly different from each other and, if not, what our best estimate for γ is. γ has a complicated distribution but, by making a few assumptions, we can get some simple results. Let us assume that the γ_i's have the same mean, μ say. However they will have different variances as p_1 and p will be different in different experiments. Let the $\text{var}(\gamma_i) = \sigma_i^2$. Then, if we assume that the γ_i's are approximately normally distributed, the maximum likelihood estimate for μ ($\hat{\mu}$, say) is given by

$$\hat{\mu} = \sum_{i=1}^{n} \frac{\gamma_i/\sigma_i^2}{\sum_{i=1}^{n} 1/\sigma_i^2} \quad (\text{where we assume } \sigma_i^2\text{'s are known}). \tag{A 5}$$

This is also the best linear estimate for all distributions of γ and thus this is a 'good' estimator. It is also unbiased (i.e. $E(\hat{\mu}) = \mu$) and its variance is $\left[\sum_{i=1}^{n} 1/\sigma_i^2\right]^{-1}$. In the very unusual case when all the variances are the same, then (A 3) simplifies to the usual formula for the sample mean, i.e.

$$\hat{\mu} = \frac{1}{n}\sum_{i=1}^{n} \gamma_i.$$

https://doi.org/10.1017/S0016672300017523 Published online by Cambridge University Press

Thus using formula (A 1), we can estimate σ_i^2 for each sample and thus get an estimate, $\hat{\mu}$, for the true value of the ratio of % steriles to % lethals. The estimation of $\hat{\mu}$ has been given before testing if all the γ_i's have the same mean, as $\hat{\mu}$ is used in the test. The standard error of $\hat{\mu}$ and approximate confidence limits can be found by using the fact that

$$\text{var}\,(\hat{\mu}) = \left[\sum_{i=1}^{n} \frac{1}{\sigma_i^2}\right]^{-1}.$$

Again, using the assumption that the γ_i's are approximately normal, it can be shown that

$$Z = \sum_{i=1}^{n} \{(\gamma_i - \hat{\mu})^2/\sigma_i^2\}$$

has a χ^2_{n-1} distribution. Thus Z can be calculated and compared at the required significance level with numbers from tables of the χ^2-distribution with $(n-1)$ degrees of freedom (n being the number of readings for γ).