KOREAN JOURNAL OF ZOOLOGY
Vol. 19, No. 1, 15—24 (1976)

Frequency and Allelism of Deleterious Genes Concealed in Korean Natural Population of Drosophila —Lethality, Sterility and Visible Mutants—

Jong-Kil Choo and Taek Jun Lee
(Dept. of Biology, Chungang University)

自然集團에 保有되어 있는 초파리 有害遺傳子의 頻度와 同座率에 關한 研究 —致死因子, 不姙因子 및 可視突然變異因子—

秋 鍾 吉·李 澤 俊
(中央大 生物學科)

(Received February 10, 1976)

摘 要

1971年부터 1973年까지 安養에서 採集한 *Drosophila melanogaster*의 第2染色體上에 保有되어 있는 致死因子, 不姙因子 및 可視突然變異體의 頻度를 調査하였다.

1) 致死 및 半致死因子를 保有하고 있는 染色體의 頻度는 3년간 평균 28.2%로 나타났고, 3年간의 빈도는 큰 差異가 없었다.

2) 安養自然集團의 致死遺傳因子間의 同座率은 0.77%였다. 致死因子로 인한 集團內에서의 elimination rate (IQ^2)는 0.0008로 추정되었다.

3) 第2染色體에 保有되어있는 劣性不姙因子는 암컷 9.1%, 수컷 6.8%, 그리고 兩性不姙이 2%로 나타났다.

4) 劣性可視突然變異體인 *rbl*/*rbl*과 *bw*/*bw*은 第2染色體에서 동형접합자가 될 때 檢出된다. 本實驗에서 *rbl* 因子는 2.7%였고 *bw* 因子는 1.3%로 나타났다.

INTRODUCTION

Deleterious genes concealed in natural populations of *Drosophila melanogaster* have been analysed by several investigators. Ives (1954) and Hiraizumi and Crow (1960) investigated on the frequency of the second chromosomes carrying the recessive lethal genes in natural populations at several localities of the United States. Genetic variablities of the South East Asian natural populations have been reported by many investigators. Minamori and his co-workers (1974) have analysed annually since 1961 on the genes of lethal, segregation distorter and deltaassociating chromosomes in Hiroshima natural population of Japan. They reported that the lethal and semilethal chromosomes through eleven years disclosed an increasing trend in frequencies and a decreasing trend in frequencies of allelism between lethal chromosomes. Similar results of such trends were observed by Oshima and his colleagues (Oshima 1965, 1968; Watanabe 1969; Watanabe and Oshima, 1969) in central populations of Japan since 1959. They reported that some lethal genes had persisted in natural populations when they were associated with an epistatic gene complex and heterotic inversions. Oshima and Choo (1972) reported that the deleterious genes concealed in natural populations of Kofu and Katsunuma of Japan increased annually from 1969 to 1972. Paik and his co-workers (Paik 1960, 1966, 1969) have reported on the genetic structures of several localities in Korean natural populations.

The frequencies of sterility genes concealed in natural populations of *Drosophila* species have been studied several years ago by many workers (Pavan *et al.*, 1951; Dobzhansky and Spassky 1953, 1954; Oshima and Choo 1972; Oshima and Watanabe 1973). They reported that the sterility in natural population was greatly influenced by environmental conditions.

Many visible mutants as well as lethal genes have been concealed in natural populations. Watanabe (1969) detected out recessive visible mutants, *rbl* (reduced bristles), on the second chromosomes which have been frequently extracted from natural populations of central Japan. Ives (1945) and Minamori and Saito (1964) also isolated the several visible mutants in American and Japanese natural populations.

The present work was undertaken to determine the genetic structure of natural population of Anyang, Korea. The population has been observed annually for three years since 1971 as to the frequencies of lethal and sterile chromosomes, and the allelism rate between lethals. The experiment also detected out the visible mutants on the second chromosomes.

MATERIALS AND METHODS

Drosophila melanogasters used in the present experiment were collected by nets at graperies in Anyang, Gyunggi prefecture about sixty kilometers from Seoul. *Drosophila melanogaster* is a dominant species as well as *D. auraria* species group at mid-autumn in this area.

1. Isolation of the second chromosome and estimation of homozygote viablity.

Several hundreds of male flies collected from natural population were randomly sampled. A second chromosome of each individual male fly was isolated by the method of completly marked inversions as shown in Fig. 1. A wild type male fly extracted from natural population was mated to three *Cy/Pm* virgin females (*Cy*,

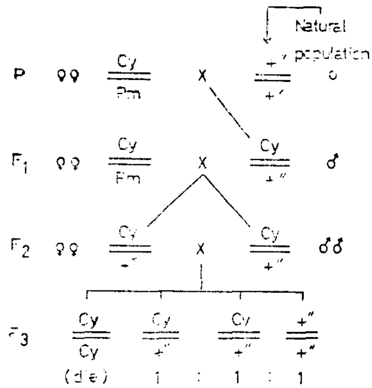

Fig.1. Mating scheme for isolating a second chromosome from natural population. +′ and +″ mean the chromosomes from natural population.

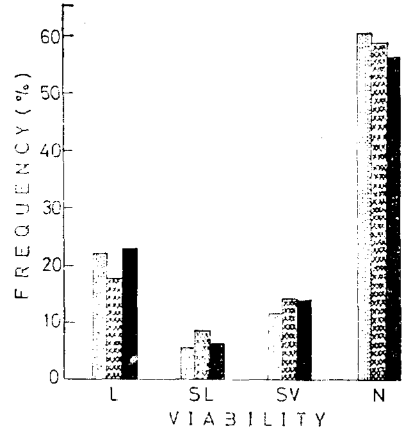

Fig.2. Frequency distribution of homozygotes for the second chromosomes isolated from Anyang natural population. Left, middle and right columns mean 1971, 1972 and 1973, respectively.

Curly wings and *Pm*, Plum eyes are dominant marker genes on the second chromosomes which are lethal when homozygote). A single F_1 male was mated again to three *Cy/Pm* virgin females. Three pairs of *Cy*/+″ females and *Cy*/+ males (+″ is a wild type from natural population) of F_2 generation were cultured in a vial. In the F_3 generation, the expected ratio of *Cy* (*Cy*/+″) to non-*Cy*(+″/+″) flies was 2 : 1 theoretically. Deviations from this 2 : 1 ratio were ascribed to genes carried by the tested chromosomes. If a second chromosome sampled from natural population has a deleterious gene, the proportion of non-*Cy* flies in the F_3 generation should be lower than 33.3%. According to the proportion of non-*Cy* flies to total emerged flies, the degree of deleterious gene could be estimated. In the present experiment, the second chromosomes were classified by proportion of non-*Cy* flies to the total emerged ones as follow; lethal (L) with 0 to 1.0%, semilethal (SL) with 1.1 to 16.7%, subvital (SV) with 16.8 to 25.0%, normal (N) with 25.1 to 42.0% and supervital (SUV) with above 42.1%, respectively.

The lethal chromosomes extracted simultaneously have been maintained by *Cy*-balanced system in the laboratory. The half diallel crosses between lethal genes were performed to detect whether they had genes allelic to each other or not.

2. Isolation of sterility genes

Male or female flies sampled from natural population were mated individually to three *Cy/Pm* females or males. When no larvae were detected for five days after crosses, the *Cy/Pm* flies were renewed and the test was continued for another five days. If no larvae were found within these ten days, the wild type fly was classified as phenotypic sterile.

In the F_3 generation of the cross test for *Cy/Pm* method (Fig.1), *Cy* and non-*Cy* flies were emerged. Non-*Cy* flies were homozygotes for a second chromosme which originated in a natural wild fly. Five non-*Cy* male flies of each line were mated with five virgin females of Oregon-R strain. After five days, when no larvae could be found, all females were changed to new virgin females and the vial was continuously cultured for next five days. The larvae could not be found in these successive crosses and the same test was repeated in the next generation to confirm the complete genetic sterility. Test for female sterility was performed by crossing with Oregon-Rundles in the same manner.

3. Isolation of visible mutants

The mating scheme to find out and isolate a visible mutant gene of the second chromosome was shown in Fig. 1. In the F_3 generation of the cross test, a wild type extracted from natural population was duplicated and deleterious effects of genes on it were revealed. Theoretically homozygous flies should emerge in the ratio one third to total flies. From the phenotype of the non-*Cy* flies, it could be ascertained whether the chromosome had a recessive visible mutant gene (*rbl* and *bw*) or not. If the chromosome had a lethal gene, we could have neither non-*Cy* flies nor a visible mutant gene in the F_3 generation.

All cultures of flies were kept in a constant temperature room at 25±1°C. The medium used in the present experiment was standard corn meal, molasses, agar type sprayed with live yeast and with a few drops of propionic acid as a mold inhibitor.

RESULTS

1. Estimation of homozygote viability and allelism between lethal chromosomes

Table 1 represents the frequencies of deleterious (L, SL and L+SL) and normal (SV and N) genes on the second chromosomes and the lethalities in the collection

Table 1. Frequency distribution of deleterious and normal second chromosomes isolated from the Anyang natural population

Year	No. of tested chromosomes	L	SL	SV	N	L+SL
1971	195	43	11	23	118	54
	(%)	(22.1)	(5.6)	(11.8)	(60.5)	(27.7)
1972	90	16	8	13	53	24
	(%)	(17.8)	(8.9)	(14.4)	(58.9)	(26.7)
1973	200	46	13	28	113	59
	(%)	(23.0)	(6.5)	(14.0)	(56.5)	(29.5)
Total	485	105	32	64	284	137
	(%)	(21.6)	(6.6)	(13.2)	(58.6)	(28.2)

periods at 1971, 1972 and 1973 were 22.1, 17.8 and 23.0 per cent, respectively. However, lethal plus semilethal chromosomes found to be similar in relative frequency at the successive collection periods. Sample size of collection in 1972 was smaller than another two years, but no significant difference was detected in the frequency of deleterious chromosomes.

All lethal chromosomes extracted from natural population were maintained by Curly-balanced system in the laboratory. The isolated lethal chromosomes were half diallelically permuted in the crosses, and the results of allelism test of each year were presented in Table 2. From the total of 1,299 crosses, the allelic relationship between lethals were found only 10 crosses. Therefore an average annual frequency of allelic crosses in the three successive years of the Anyang natural

Table 2. The allelism test between lethals isolated from the Anyang natural population

Year	No. of lethals isolated	No. of crosses	No. of allelic crosses	allelism rate (%)
1971	43	903	5	0.55
1972	16	120	1	0.83
1973	24	276	4	1.45
Total	83	1299	10	0.77

population was estimated to be 0.77%. However, the allelic frequency between lethals isolated in 1973 seemed to be increased trend as comparing to the past two years.

The analysis of the relations between the frequency of the appearance of lethal heterozygotes and the frequency of their spontaneous origin through mutation can be estimated by the selection rate of lethal heterozygotes in a natural population. Thus, if the frequency of lethal chromosomes in a population is Q and frequency of alleles among the lethals is I, a fraction IQ^2 of the zygotes in this population will die on account of homozygous for lethals. The results of IQ^2 values in the present data were shown in Table 3. The average IQ^2 in the Anyang natural population through three years estimated to be 0.0008.

Table 3. Elimination frequencies (IQ^2) of deleterious chromosomes due to homozygosis in the Anyang natural population

Year	Frequency of L+SL (Q)	Frequency of allelism (I)	IQ^2
1971	0.2769	0.0055	0.0004
1972	0.2667	0.0083	0.0006
1973	0.2950	0.0145	0.0013
Mean	0.2825	0.0094	0.0008

2. Isolation of sterility genes

The annual frequencies of sex-specific and both sexes sterile chromosomes concealed in the Anyang natural population were presented in Table 4. The mean

Table 4. Frequency of recessive sterile genes isolated from the Anyang natural population

Year	No. of tested chromosomes	Female	Male	Both sexes	Total
1971	148	23	12	2	37
	(%)	(15.5)	(8.1)	(1.4)	(25.0)
1972	66	4	6	2	12
	(%)	(6.1)	(9.1)	(1.9)	(18.2)
1973	138	5	6	3	14
	(%)	(3.6)	(4.3)	(2.2)	(10.1)
Total	352	32	24	7	63
	(%)	(9.1)	(6.8)	(2.0)	(17.9)

frequency of sterile flies among heterozygotes seemed to be a range of 3 to 10 % in males or females. In 1971, however, the female sterile chromosomes observed higher frequency than the males as about two times. On the other hand, sterile chromosomes detected on the both sexes have shown lower frequency than the sex specific sterile genes.

3. Isolation of visible mutants

The spontaneous mutant genes of *bw* (brown) and *rbl* (reduced bristles) were first

Table 5. Frequencies of visible mutants (*bw*, *rbl*) isolated from the second chromosomes in the Anyang natural population

Year	No. of tested chromosomes	*bw*	*rbl*
1971	148	3	6
	(%)	(2.0)	(4.1)
1972	74	1	2
	(%)	(1.3)	(2.7)
1973	154	1	2
	(%)	(0.6)	(1.3)
Total	376	5	10
	(%)	(1.3)	(2.7)

discovered by Waller in 1921 (Lindsley and Grell, 1967) and Watanabe in 1964 (Watanabe, 1959 b), respectively. Frequencies of recessive *bw* genes concealed in the Anyang natural population observed fairly low as 1.3% on the average for three years. However, represents of *rbl* genes of the same population were 2.7% on the average frequency. In the total 376 tested chromosomes, the recessive mutant genes of these *bw* and *rbl* on the second chromosomes were found in fifteen flies.

DISCUSSION

The data presented in this paper show a general pattern of annual changes in the frequency of lethal chromosomes concealed in Korean natural population of *Drosophila melanogaster*. There is only a small difference between frequencies of lethal chromosomes found in fall collection of successive years. Comparing to the lethal frequency and the allelism rate between lethals in another natural populations of Korea and those of Japanese populations were interest to know on the genetic structures of natural variations. In Korean natural populations, Paik (1960, 1966) reported that the combined frequency of L+SL chromosomes ranged from 7.5 to 16.7% among the second chromosomes, and their allelism rate was comparatively high within population in several localities of Korea. Oshima and Choo (1972) and Minamori *et al.* (1974) reported that the frequency of deleterious chromosomes appeared an increasing trend from a few years ago at populations of Hiroshima and Kofu-Katsunuma of Japan. The reason for this increase of lethality in natural populations were not yet known. The frequency of lethal chromosomes and allelism rate between lethals in the present data were estimated to be 21.6% and 0.77% on the average for three years since 1971. The sample size of the collection area was the important factor for the frequency of homozygote viability in the population. Ecological habitats would be controlled by the genetic structures of the population. The Anyang district located at central part of Korean peninsular is the largest orchard comparing to other localities of Korea.

Oshima and his colleagues (Oshima 1958, Oshima 1968, Oshima and Choo 1973, Watanabe and Oshima 1969) observed annually that the genetic variabilities in Japanese natural populations, and the frequency of the elimination caused by deleterious genes (IQ^2) in homozygotes was calculated to be 0.0016 on the average from 1965 through 1968 at Katsunuma natural population (Watanabe 1969 a). IQ^2 caused by deleterious chromosomes in the present investigation was estimated to be 0.0008 on the average for three years, and it was quite the same value as Hiroshima natural population of Japan (Minamori *et al.*, 1974). From the results of homozygote viability and elimination rate on the second chromosomes, the genetic structure of the Anyang natural population would be maintained as the present state when environmental effects were not changed.

Oshima and Watanabe (1973) reported that the sterile genes concealed in natural populations of *Drosophila melanogaster* observed about 3 or 4 % on the average, and the frequency of sterile flies was the same in male and female. They suggest that the sterile genes were associated with the low viability due to the pleiotropic effects. Sweet and Spiess (1962) have found that the sterile factors were present to exist in the population as about 4.5 %. However, Hoenisberg *et al.* (1969)

observed that the sterile genes in wild flies were zero to 9 % at several Colombian natural populations of the United States. According to the results of several investigations, about 3 or 4 % of wild flies seemed to be sterile in natural population. In the present experiment, the frequency of sterile chromosomes of wild flies was estimated to be a range from 3 to 9 % except survey in females in 1971. Thus, the Anyang natural population of Korea was similar to Colombian or Katsunuma natural populations in the frequency of the sterile genes on the second chromosomes. However, factors caused by genes of sterility in both sexes showed a lower frequency than those for the other populations.

Watanabe (1969 b) reported that the frequency of *rbl* gene in Japanese populations varied from 3.1 to 7.1% on the average for three years (1965 to 1967). He found that *rbl* homozygotes had a low viability, and *rbl* heterozygotes manifested neutral in viability. In the Anyang population, *rbl* gene expresses low frequency comparing to those for Japanese population. Some of the other kinds of mutant genes caused by homozygote on the second chromosomes were frequently found. Among these visible mutant genes, *bw* gene observed about 1 % in the Anyang natural population.

SUMMARY

The frequency of the second chromosomes bearing deleterious genes in the Anyang natural population of Korea in *Drosophila melanogaster* was repeatedly estimated during the period from 1971 through 1973.

1) The frequency of lethal and semilethal chromosomes was calculated to be 28.2 %, and the frequencies were maintained without fluctuation for three years.

2) Allelism rate between lethal genes isolated from each year was 0.77% on the average. The rate of elimination of lethal genes (IQ^2) was estimated to be 0.0008.

3) The frequency of sterile gene on the second chromosomes was estimated to be 9.1% for females, 6.8% for males and 2.0% for both sexes, respectively.

4) Recessive visible mutant genes, namely *rbl* and *bw* genes, were frequently extracted when the chromosomes were revealed in homozygous. The frequencies of these mutants were found to be 1.3% for *bw* genes and 2.7% for *rbl* genes, respectively.

ACKNOWLEDGEMENT

The authors are indebted to Drs. Chozo Oshima and Takeo K. Watanabe, the National Institute of Geneitcs, Japan for their valuable suggestions and criticisms thronghont the course of this investigation.

REFERENCES

Dobzhansky, Th. and B. Spassky, 1953. Genetics of natural populations. XXI. Concealed variability in two sympatric species of *Drosophila*. *Genetics* **38** : 471—484.

Dobzhansky, Th. and B. Spassky, 1954. Genetics of natural populations. XXII. A comparison of the concealed variability in *Drosophila prosaltans* with that in other species. *Genetics* **39** : 472—487.

Hoenisberg, H.F., L.E. Castro and L.A. Granobles, 1969. Population genetics in the American tropics. V. The sterility content in the second chromosomes of *Drosophila melanogaster* from Fusagasuga, Colombia. *Genetics* **40** : 543—554.

Hiraizumi, Y. and J.F. Crow, 1960. Heterozygous effects on viability, fertility, rate of development and longevity of *Drosophila* chromosomes that are lethal when homozygous. *Genetics* **45** : 1071—1083.

Ives, P.T., 1945. The genetic structure of American populations of *Drosophila melanogaster*. *Genetics* **30** : 167—196.

Lindsley, D.L. and E.H. Grell, 1967. "Genetic variation of *Drosophila melanogaster*" Carnegie Institute Press.

Minamori, S. and Y. Saito, 1964. Local and seasonal variations of lethal frequencies in natural populations of *Drosophila melanogaster*. *Japan. J. Genetics* **38** : 290—304.

Minamori, S., K. Ito, A. Nakamura, Y. Ando, and H. Shiomi, 1974. Increasing trend in frequencies of lethal and semilethal chromosomes in a natural population of *Drosophila melanogaster*. *Japan. J. Genetics* **48** : 41—52.

Oshima, C., 1965. Persistence of some recessive lethal genes in natural populations of *Drosophila melanogaster*. Mutation in population. Proc. Symp. Mutational Process, Prague, 41—48.

Oshima, C., 1968. Persistence of some recessive lethal genes in natural populations of *Drosophila melanogaster*. *Proc. 12th Intern. Congr. Genet.* **2** : 170—171.

Oshima, C. and J.K. Choo, 1972. Deleterious and sterility genes in Japanese and Korean natural populations of *Drosophila melanogaster*. *Ann. Report of Natl. Inst. Genet.* **22** : 90—91.

Oshima, C. and J.K. Choo, 1973. Allelism between sterility genes in Japanese and Korean natural populations of *Drosophila melanogaster*. *Ann. Report of Natl. Inst. Genet.* **23** : 98—100.

Oshima, C. and T.K. Watanabe, 1973. Fertility genes in natural populations of *Drosophila melanogaster*. I. Frequency, allelism and persistence of sterility genes. *Genetics* **74** : 351—361.

Paik, Y.K., 1960. Genetic variability in Korean natural populations of *Drosophila melanogaster*. *Evolution* **14** : 293—303.

Paik, Y.K., 1966. Genetic variabilities in second and third chromosomes from Korean natural populations of *Drosophila melanogaster*. *Japan. J. Genetics* **41** : 325—333.

Paik, Y.K. and K.C. Sung, 1968. Behavior of lethals in *Drosophila melanogaster* populations. *Japan. J. Genetics* **44**(1) : 180—192.

Pavan, C., A.R. Cordeiro, N. Dobzhansky, Th. Dobzhansky, C. Malogolowkin, B. Spassky and M. Wedel, 1951. Concealed genetic variability in Brazilian populations of *Drosophila willistoni*. *Genetics* **36** : 13—30.

Sweet, E.E. and E.B. Spiess. 1962. Frequency of sterility in a laboratory population of *Drosophila melanogaster*. *Genetics* **47** : 1519—1534.

Watanabe, T.K., 1969 a. Frequency of deleterious chromosomes and allelism between lethal genes in Japanese natural populations of *Drosophila melanogaster*. *Japan. J. Genetics* **44** : 171—187.

Watanabe, T.K., 1969 b. Persistence of a visible mutant in natural populations of *Drosophila melanogaster*. *Japan. J. Genetics* **44** : 15—22.

Watanabe, T.K. and C. Oshima, 1969. Persistence of lethal genes in Japanese natural populations of *Drosophila melanogaster*. *Genetics* **64** : 93—106.