HETEROZYGOTE VIABILITY OF A SECOND CHROMOSOME RECESSIVE LETHAL IN DROSOPHILA MELANOGASTER[1]

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THE present study is concerned with the second chromosome recessive lethal *l(2)55i* in *Drosophila melanogaster*. This gene has been studied previously by MUKAI and BURDICK (1959) who showed that single gene heterosis is associated with this particular lethal. In the previous work, four experimental populations, differing with respect to initial lethal gene frequency and genetic background, were established. In order to estimate the viability of the lethal heterozygotes compared to the wild type homozygotes, these populations were sampled for several generations and the frequency of lethal heterozygotes in each generation determined. These frequencies were then compared to the frequencies which would have been expected if the lethal heterozygote had shown no selective advantage over the wild type. The frequency of lethal heterozygotes in the experimental populations reached an equilibrium point of about 0.42.

Various factors relating to the maintenance of *l(2)55i* in populations will be considered in this paper. How is the heterotic effect expressed? Do lethal heterozygous females lay more eggs than the wild type females? Do lethal heterozygous males in competition with wild type males have an advantage in mating ability? Do lethal-carrying sperm have a competitive advantage over sperm carrying the wild type allele? Is zygotic viability of lethal heterozygotes higher than that of wild types? What is the relative importance, or what is the contribution, of each of these four factors to the maintenance of *l(2)55i* at such a high frequency?

Other factors of interest concerning the gene *l(2)55i* are presented including the present estimate of the lethal heterozygote frequency in the stock from which the gene was originally extracted, present estimate of the frequency of lethal heterozygotes in the four experimental populations, and time of lethality of the gene in homozygous condition.

MATERIALS

The following stocks were developed by BURDICK and his collaborators:

W-1 is a wild type stock collected at Erie, Pa. in the fall of 1954 and maintained by random mating.

W-11 is a wild type stock inbred from A. E. Bell's stock, CP.

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W-109 is a homozygous wild-type stock extracted by the CMI technique (BURDICK 1954a) from W-11.

C-101 has the second chromosome lethal *l(2)55i* balanced over *Ins(2)SM1*, *al*^{2} *Cy sp*^{2} with first, third and fourth chromosomes homozygous. This stock and W-101 were extracted from a single W-1 female by the CMI technique. W-101 is a completely homozygous stock. Both C-101 and W-101 have been maintained by random mating.

The following populations were established by MUKAI:

Population 1 was established by 50 pairs of lethal heterozygotes (obtained as virgin offspring from a cross between C-101 and W-101) in each of seven half-pint bottles. The genetic background of the lethal heterozygotes in this population is that of a single W-1 female.

Population 2 was established in the same way as Population 1 except that the lethal heterozygotes were obtained from a cross between C-101 and W-109. The genetic background for the lethal heterozygotes in Population 2 is 50 percent W-1 and 50 percent W-109.

Population 1(3–4) has no second chromosome lethal and was established from a single pair mating of lethal-free flies in the 22nd generation of Population 1. Therefore, the genetic background of this population is that of Population 1. This stock is maintained by random mating.

Population 3 consists of eight bottles. At its origination, 50 pairs of lethal-free flies from Population 1(3–4) and lethal heterozygous flies from Population 1 were put into each bottle. The expected frequency of lethal heterozygotes in the original 100 flies was 0.05. The genetic background of this population is that of Population 1.

Population 4 consists of ten bottles and was established in the same manner as Population 3 except that the lethal-free flies came from Population 2(1–4) (established by a single pair mating of lethal-free flies in the 17th generation of Population 2) and the lethal heterozygotes came from Population 2. The expected frequency of the lethal heterozygote in the starting generation of Population 4 was 0.05. The genetic background of this population is that of Population 2.

Populations 1, 2, 3 and 4 were maintained by MUKAI for the first 15 generations in the following manner: A random sample of 50 pairs of flies from each bottle was transferred to a new bottle every 15 days. In order to avoid intergeneration mating, all flies were removed from the bottles nine days after transfer. After 15 generations, each bottle of each population was maintained by mass transfer every 15 days with no precautions taken to avoid intergeneration matings.

All stocks were maintained and all experiments carried out at a temperature of 25 ± 1°C. The culture medium was that described by BURDICK (1954b). Active dry yeast was added to the bottles at the time of use.

Time of action of homozygous l(2)55i

The following procedure was used to determine the time of action of homo-

zygous *l(2)55i*. A cross between Population 1(3–4) and C-101 yields two types of offspring, as follows:

$$+/+ \times Cy/l \longrightarrow +/l \text{ and } +/Cy$$

Ten pairs of $+/l$ flies resulting from such a cross were randomly selected and put into a bottle. Ten pairs of $+/+$ flies from Population 1(3–4) were placed in another bottle for use as a control. When the flies had been in the bottles for 24 hours, each group of 20 flies was transferred to an egg-laying chamber similar to that described by KING (1955). The two egg-laying chambers were placed on medium in Petri dishes. Charcoal was added to the medium in order to facilitate egg counting. Immediately before placing the egg-laying chamber on the food, the surface of the food was seeded with a solution of acetic acid, water and active yeast.

Flies were allowed to deposit eggs on the medium for approximately 12 hours. After 12 hours the egg-laying chambers were transferred to new medium. This procedure was repeated until four groups of eggs were obtained from each of the two types of matings. A total of 1,597 eggs was included in the experiment.

The following counts were made for each of the eight groups of eggs: (1) number of eggs deposited, (2) number of eggs which hatched into larvae, (3) number of larvae which pupated, and (4) number of pupae which developed into adult flies.

One fourth of the zygotes from the mating $+/l \times +/l$ were expected to be homozygous for the lethal gene and, therefore, were expected to die at some time during development.

The analysis of variance procedure was applied to the larvae/egg, pupae/larvae and imago/pupae ratios. In no case was there a significant difference between groups of eggs within mating types. There was a highly significant difference between mating types in the pupae/larvae ratios, but no difference between mating types in the larvae/egg or imago/pupae ratios. The analysis for pupae/larvae ratios is given in Table 1. It was assumed that there was no interaction between groups and mating types.

The four groups of each mating type were pooled in order to estimate the relative pupation rate of offspring of the cross $+/l \times +/l$. The results are shown in Table 2.

TABLE 1

Analysis of variance in pupae/larvae ratios

Source	d.f.	Mean square	F
Among groups	3	23.7087	2.5289
Between mating types	1	1002.3040	106.9147**
Error	3	9.3748	
Total	7		

** Significant at the 0.01 level.

TABLE 2

Numbers of larvae and pupae produced from two types of matings in the test for time of lethality

	Type of mating	
	$+/l \times +/l$	$+/+ \times +/+$
Larvae	678	504
Pupae	481	469
Pupae/larvae ratio	0.7094	0.9306
Relative pupation rate = 0.7094/0.9306 = 0.7623		

If this lethal produced its effects solely in the larval stage, a relative pupation rate of 0.75 would be expected. The relative pupation rate of 0.7623 is sufficiently near 0.75 to conclude that homozygous *l(2)55i* acts during the larval period by preventing larvae from making the physiological changes necessary for development into the pupae stage.

Frequency of l(2)55i *heterozygotes in W-1*

The gene *l(2)55i* was first found in, and extracted from, the W-1 (Erie) wild type stock. At that time the frequency of the lethal heterozygotes was estimated at 0.34 (BURDICK and MUKAI 1956). This stock was retested to determine the frequency of the lethal at the present time.

Males were randomly selected from the W-1 population and mated individually to C-101 females. These matings were one of the following two types:

1. $Cy/l \times +/+ \longrightarrow$ 50 percent Curly and 50 percent wild type.
2. $Cy/l \times +/l \longrightarrow$ 67 percent Curly and 33 percent wild type.

Therefore, if in the offspring of these single male matings a ratio of 1 Curly:1 wild type was found, the male parent was scored as not carrying the lethal gene; if the ratio was 2 Curly:1 wild type, the male parent was scored as a lethal heterozygote. In those cases where there were insufficient offspring and/or a χ^2 discrimination could not be made between the two ratios, the male in question was disregarded.

One hundred eight males from the W-1 population were tested simultaneously for the presence of the lethal gene. A total of 11,042 offspring was counted. Six of the males were disregarded due to the afore-mentioned reasons. Of the remaining 102 tested males, 24 were heterozygous for the lethal gene. This yields an estimate of $Q = 0.2353 \pm 0.0420$ for the W-1 population, where $Q =$ the frequency of lethal heterozygotes.

The frequency of *l(2)55i* has, therefore, changed very little in three years ($Q = 0.34$ as compared to $Q = 0.2353 \pm 0.0420$) and has been maintained in this population under random mating conditions for more than four years.

Frequency of l(2)55i *heterozygotes in the four experimental populations*

The frequency of *l(2)55i* heterozygotes in each of the four experimental populations was determined. In the generation before testing was to begin, 50 pairs of

flies were selected at random to begin the next generation. For the following five generations, 50 pairs were transferred every 15 days and at the time of transfer 15 males from each bottle were tested for the presence of the lethal. The testing method was the same as that described for testing the W-1 males. All flies were removed from each population bottle on the eighth day after introduction. Each bottle was analyzed as a possible subpopulation of the original population.

Population 1: Population 1 consists of seven bottles. Fifteen males from each bottle were tested in generations 57, 58, 59, 60 and 61. A total of 525 males was tested and 65 were disregarded due to insufficient offspring and/or the inability to distinguish between a 1:1 and 2:1 ratio.

The analysis of variance technique was applied to the estimates for Q for each bottle in each generation. No significant differences in lethal heterozygote frequency (Q) were found between bottles or between generations. A single estimate of the frequency of lethal heterozygotes in Population 1 was obtained. Of the total 460 males, 88 were scored as being lethal heterozygotes. This gives an estimate of $Q = 0.1913 \pm 0.0194$ in Population 1 as shown in Table 3.

Population 2: Population 2 consists of seven bottles. Fifteen males from each bottle were tested in generations 46, 47, 48, 49 and 50. A total of 525 males was tested and 117 were disregarded due to insufficient offspring and/or the inability to distinguish between a 1:1 and 2:1 ratio.

This population was analyzed in the same way as Population 1. There was no significant difference in lethal heterozygote frequency between generations, but there was a highly significant difference between bottles. Because of this, an estimate of Q for Population 2 as a whole was not calculated. Estimates of Q for each of the seven subpopulations were obtained from the means of the five tested generations. These estimates are given in Table 3.

Population 3: Population 3 consists of eight bottles. Fifteen males from each bottle were tested in generations 33, 34, 35, 36 and 37 and, of the 600 males tested, 70 were disregarded due to insufficient offspring and/or inability to distinguish between a 1:1 and 2:1 ratio.

In this population there was no significant difference in Q between bottles, but there was a significant difference between generations. This difference between generations was quite irregular and was in no way associated with a trend. Because of this, the difference was attributed to sampling variation and an estimate of Q was made for Population 3 as a whole. Of the 530 males tested, 117 were heterozygous for the lethal. This yields an estimate of $Q = 0.2208 \pm 0.0195$ (Table 3).

Population 4: Population 4 consists of ten bottles. Fifteen males from each bottle were tested in generations 27, 28, 29, 30, and 31 and, of the 750 males tested, 146 were disregarded due to the afore-mentioned discrimination difficulties.

As in Population 2, there was no difference in lethal heterozygote frequency between generations, but there was a highly significant difference between bottles. An estimate of Q for the entire population was not made. Estimates of the

TABLE 3

Summary of information concerning Populations 1, 2, 3, 4 and W-1

	Genetic background	Lethal heterozygote freq. in starting generation	Est. equilibrium freq. gen. 7–16	Equilibrium freq. of subpopulations as est. in this study	Pooled est. of equilibrium freq. with respect to subpop. and generations
Pop. 1	W-1	100%	.420 ± .190	.100 ± .028 .138 ± .055 .167 ± .053 .203 ± .077 .209 ± .014 .219 ± .042 .312 ± .035 (Gen. 57–61†)	.191 ± .019
Pop. 2	W-1 50% W-109 50%	100%	.424 ± .017	.179 ± .040 .189 ± .096 .241 ± .038 .262 ± .036 .317 ± .055 .333 ± .094 .508 ± .058 (Gen. 46–50†)	.....
Pop. 3	Pop. 1	5%*	.....	.156 ± .035 .167 ± .024 .177 ± .040 .227 ± .052 .228 ± .064 .254 ± .067 .262 ± .061 .286 ± .083 (Gen. 33–37†)	.221 ± .019
Pop. 4	Pop. 2	5%*	.....	.129 ± .049 .143 ± .026 .164 ± .078 .246 ± .088 .300 ± .060 .339 ± .044 .441 ± .046 .441 ± .065 .569 ± .067 .571 ± .106 (Gen. 27–31†)	.....
W-1	.....	....	.....	.....	.235 ± .042

* Expected frequency.
† Each estimate is the mean of tests of these five generations.

frequency of the lethal heterozygote based on the means of the five tested generations for each of the ten subpopulations of Population 4 are given in Table 3.

Information about the four experimental populations and W-1 is summarized in Table 3. The four experimental populations differed as to genetic background

and lethal frequency in the starting generation. Populations 1 and 3 are similar in genetic background and Populations 2 and 4 are similar in genetic background. Populations 1 and 2 began with 100 percent lethal heterozygote ferquency whereas Populations 3 and 4 have an expected frequency of five percent at their origination. The results indicate that an important factor influencing lethal heterozygote frequency in these populations is the genetic background.

Populations 1 and 3 were derived from a single W-1 female and contain only the heterogeneity present in that female plus any spontaneous mutations which might have occurred since Population 1 was established. There were no heterogeneities between bottles in either of the populations with the W-1 genetic background.

Populations 2 and 4, on the other hand, are 50 percent W-1 and 50 percent W-109 in genetic background and would, therefore, be expected to have been more heterogeneous in the starting generations. Evidence of genetic difference is indicated by the fact that in both of these populations there were heterogeneities among the various subpopulations. This is to be expected since the greater amount of heterozygosity present in these populations allows for a greater chance of genetic segregation of genes other than the lethal. Therefore, the genetic background might be somewhat different from bottle to bottle in these populations. But, the lethal has not been lost, nor has it even reached a very low frequency in any subpopulation.

A t test was applied to the observed difference between Q of Population 1 (0.191) and Q of Population 3 (0.221). This difference was found to be not significant. A pooled estimate of the frequency of *l(2)55i* in these two populations with W-1 background yielded an estimate of $Q = 0.2071 \pm 0.0138$. The pooled estimates of $Q \pm$ one standard deviation in Populations 1 and 3 and in the W-1 stock overlap this value.

Factors involved in the expression of heterosis

Studies concerning the various factors involved in the expression of overdominance of the gene *l(2)55i* have been carried out using Population 1(3–4), a derivative of Population 1. The results, therefore, apply only to populations with the W-1 background.

Zygotic viability: Evidence that there is no difference in zygotic viability (hatchability + larval competitive ability + length of larval period) between the wild type flies and flies heterozygous for *l(2)55i* was presented by MUKAI and BURDICK (1959). The relative zygotic viability of lethal heterozygotes was estimated by them at 0.974 ± 0.032 when Population 1(3–4) was used in the experiment. This result, then, applied to Populations 1 and 3. Additional evidence suggesting equal viability is the fact that in Population 1, starting at $Q = 1.00$, the second generation value of $Q(Q = 0.673)$ approximates the expected value $(Q = 0.667)$ based on no difference between the two types of flies in either zygotic viability or in sperm competitive ability.

Sperm competitive ability: Evidence indicating no difference between *l* and + sperm in sperm competitive ability (*s*) was cited in connection with zygotic viability. MUKAI and BURDICK (1959) also showed no difference in sperm competitive ability with direct experimentation. *Cy/l* females were mated to +/*l* males and an almost perfect 2:1 ratio of Curly to wild type was obtained in the offspring.

The possibility that *l*-carrying sperm might have a competitive advantage over +-carrying sperm was retested in this study.
Two types of crosses were made as follows:

A		B	
+/+ × *Cy/l*		+/+ × *Cy/+* (from +/+ × *Cy/l*)	
↓		↓	
Cy/+ and +/*l*		*Cy/+* and +/+	
(a)	(b)	(a)	(c)

Because there is no difference between +/+ and +/*l* flies in zygotic viability, a comparison of the relative proportion of flies in the (b) and (c) classes of offspring will provide information concerning sperm competitive ability. Ten bottles (3-pair matings) of each of the two crosses were made. The offspring were counted daily for six days beginning on the tenth day.

A χ^2 test for heterogeneity was applied to the data. The test indicated that the ten bottles within each cross were homogeneous. The pooled totals for each of the crosses are given in Table 4. The difference between the (b) and (c) classes is not significant. We can conclude that there is no difference between the competitive ability of *l*- and +-carrying sperm.

Female fecundity: Higher female fecundity may be responsible for the heterosis exhibited by *l(2)55i*. The fecundity of +/*l vs.* +/+ females was tested. Population 1(3–4) females were mated to C-101 males to obtain +/*l* males and females. The possibility that the type of male (+/+ or +/*l*) might affect fecundity was considered. The following four types of matings were made:

1. +/+ × +/+
2. +/+ × +/*l*
3. +/*l* × +/+
4. +/*l* × +/*l*

TABLE 4

Pooled data for test of sperm competitive ability

Mating			
+/+ × *Cy/l* Offspring		+/+ × *Cy/+* Offspring	
Cy/+ (a)	*l/+* (b)	*Cy/+* (a)	+/+ (c)
1245	1250	1314	1300
$\frac{(b)}{(a)+(b)} = 0.50100$		$\frac{(c)}{(a)+(c)} = 0.49732$	
$s = 1.0074$		$s = 1.0000$	

One female and two males were placed in each of 20 egg-laying bottles for each of the four crosses. Therefore, one replication of the experiment consisted of 80 egg-laying bottles and there were five replications. Two hundred +/+ and 200 +/*l* females were tested.

In the four experimental populations, the average effective egg-laying time was from age 60 hours to age 180 hours. In the test of fecundity the average age of females transferred to egg-laying caps was six hours. Any eggs deposited between this time and 48 hours later were not considered. Individual egg counts were made every 24 hours beginning on the third day and ending on the eighth day (effective egg-laying time from 54 to 198 hours). Fifty-two of the original 400 females were discarded for the following reasons: (1) Some of the females died. (2) Some lived but laid very few eggs. The fact that some laid very few eggs could have been due to either overetherization or a genetical inability to produce and/or deposit eggs. (3) Some of the females lived and deposited a high number of eggs in the early days but failed to lay in the later days of the experiment. This could have been due to an etherization effect, physical injury caused during cap transfer, or could have been a function of the genotype of the female.

The distribution by genotype of the discarded females is given in Table 5. Evidence in Table 5 indicates that approximately the same total number of each type of female was discarded but that the reasons for discarding were not similar for the two types of females. Eleven +/+ females were discarded because they laid very few eggs during the six days of count, but only two +/*l* females were discarded for this reason.

The egg-laying data were compiled on the basis of total eggs per female. Since the number of surviving females varied from cross to cross, the data were analyzed on the basis of the mean number of eggs per female for each of the four crosses within each replication. In order to test for an interaction between replication and type of mating, an error variance was calculated from the original per-female totals and corrected for use with the mean analysis. The analysis of the data is given in Table 6.

The mean square for type of female was highly significant. The mean square for interaction between type of female and replication was also highly significant. Because of the significance of this interaction, a single estimate of relative female

TABLE 5

Distribution by genotype of females discarded in test of fecundity

Reason*	Type of female +/+	+/*l*
1	13	19
2	11	2
3	1	6
Total	25	27

* See text.

TABLE 6

Analysis of variance in fecundity

Source	d.f.	Mean square	F
Replications	4	3,628.98	
Type mating	(3)	(9,587.18)	
A. Female	1	28,656.23	18.268**
B. Male	1	4.79	
C. Female × male	1	100.52	
Replication × type mating	(12)	(1,568.63)	
A. Rep. × female	4	3,729.75	12.900**
B. Rep. × Male	4	133.14	
C. Rep. × female × male	4	843.00	2.928*
Total	19		
Error (females/types/rep)† (corrected by use of harmonic mean of sample size)	328	278.8150	

** Significant at the 0.01 level.
* Significant at the 0.05 level.
† Corrected by use of harmonic mean of sample size.

fecundity was not made. No apparent difference existed between the performance of the female when mated to the wild type and the performance of the female when mated to the lethal heterozygote. Therefore, the type of male was disregarded and mean values of number of eggs were calculated for the two types of females. These mean values are given in Table 7.

The data indicate that the highest estimated value of relative fecundity (f) of $+/l$ females ($+/+ = 1$) is 1.4030 and that the lowest estimate is 1.0192. Replications 1 and 3 yielded similar values of about 1.00 and replications 2, 4 and 5 gave values approximating 1.35. An explanation for this difference among replications cannot be made at this time. A total of 175 $+/+$ females produced 60,916 eggs. The mean number of eggs per $+/+$ female was 348.0914. The mean number of eggs per female for 173 $+/l$ females laying 72,881 eggs was 421.2774. The over-all f-value for $+/l$ females was 1.2102.

Since zygotic viability and sperm competitive ability were eliminated as possible factors influencing the heterotic effect of *l(2)55i*, the relationship of f, m,

TABLE 7

Mean values of number of eggs in five replications of four types of matings

Replication	Female $+/+$ (a)	Female $+/l$ (b)	Relative fecundity (b)/(a) = f
1	338.46	347.69	1.0273
2	350.86	460.78	1.3133
3	366.97	374.03	1.0192
4	331.62	465.25	1.4030
5	356.35	479.33	1.3451

and Q were considered. According to formulae derived by MUKAI and BURDICK (1959), the following relationships exist where: m = relative mating ability of $+/l$ males, that of $+/+$ males being 1; f = relative fecundity of $+/l$ females, that of $+/+$ females being 1, and Q = equilibrium frequency of lethal heterozygotes.

$$m = [4(1-Q)^2 + 2(1-Q)(2Q-1)f] \,/\, [2(1-Q)(1-2Q) + Q(2-3Q)f] \quad (1)$$

If $m = 1$ and only fecundity is involved,

$$f = [2(1-Q)] \,/\, [Q^2 - 4Q + 2] \quad (2)$$

If $f = 1$ and only mating ability is involved,

$$m = [2(1-Q)] \,/\, [Q^2 - 4Q + 2] \quad (3)$$

The estimated Q for populations with W-1 genetic background is 0.2071 ± 0.0138. If 0.2071 is a true equilibrium and only one factor is involved, f (or m) must be equal to 1.3057 according to Formulae (2) and (3). Estimated values of f and theoretical values of m are given in Table 8. Column 1 in the table shows the pooled minimum and single maximum values of f derived from this experiment. Column 2 indicates the theoretical value of Q which the corresponding f value will support, assuming $m = 1$ (Formula 2). Column 3 shows the actual value of Q as estimated from populations with W-1 background in the previous experiments. Column 4 shows the theoretical value of m when Columns 1 and 3 are assumed true (Formula 1).

Male mating ability: The relative mating ability of $+/l$ males ($+/+$ males = 1) was tested in this study. From a cross of Population 1 (3–4) and C-101, $+/l$ and $+/Cy$ males were obtained. The following two types of crosses were made.

A

$+/+ \times (+/l$ and $+/Cy)$

$\downarrow$

$(+/+$ and $+/l)$ and $+/Cy$

B

$+/+ \times (+/Cy$ and $+/+)$

$\downarrow$

$+/+$ and $+/Cy$

The $+/+$ flies in both crosses were from Population 1 (3–4). Since it was indicated that there was no difference between $+/+$ and $+/l$ in zygotic viability nor in sperm competitive ability, mating ability (m) is the only effective factor difference in the above experiment.

Fifty females and 25 of each type of male were put into each of ten bottles. Five bottles were of mating A and five were of mating B. If the proportion of phenotypically wild type flies (a) from mating A is compared to the proportion of wild type flies (b) from mating B the result (a)/(b) is a function of relative

TABLE 8

Estimated f *and corresponding values of* m *and* Q

(1) f	(2) Calculated Q when $m = 1$	(3) Estimated Q	(4) Theoretical value of m
(min.) 1.2102	0.1587	0.2071	1.0910
(max.) 1.4030	0.2470	0.2071	0.9112

mating ability of $+/l$ males when mating ability of $+/+$ males = 1. One bottle from mating B was discarded.

There were no heterogeneities among bottles in mating A, but there were heterogeneities among bottles in mating B. A possible explanation for the above is the fact that in mating A the two types of males were the result of a single etherization whereas in mating B the two types of competing males were the result of two separate etherizations. Five single estimates and a pooled estimate of (a) and four single estimates of (b) are given in Table 9.

The most interesting comparison in this data is a comparison of the pooled estimate of (a) with the lowest estimate of (b), $m = 1.0289$. A significant difference in these two values (0.7628 and 0.7414) would suggest that the mating ability of $+/l$ males was better than that of $+/+$ males. A test indicated no significant difference between the two values. Therefore, the mating ability of $+/l$ flies is probably not greater than that of $+/+$ flies. If anything, the data show poorer mating ability of $+/l$ males.

The earlier equilibrium of 0.42 reported by MUKAI and BURDICK (1959) was evidently supported by superior female fecundity (f) and male mating ability (m) of lethal heterozygotes. Now, m appears to have dropped to 1.0 and the equilibrium has been reduced to 0.20. If we assume that m and f have experienced proportionate reductions, we can estimate that, at the time when equilibrium was 0.42, f was 1.70 and m was 1.44.

Changes in equilibrium frequency of lethal heterozygotes

As can be noted in Table 3, MUKAI and BURDICK (1959) found that their lethal heterozygous populations (Populations 1, 2, 3, and 4) reached an equilibrium where the relative frequencies of lethal heterozygotes (Q) were all about 0.42. The data presented in this report clearly show a lower equilibrium of lethal heterozygotes (about 0.20). This decrease, which took place over a period of 16 to 40 generations, may be due to the effect of mutations in the genetic background modifying the relative viability of lethal heterozygotes. Since the superviability of the lethal heterozygote is attributable to only one factor, female fecundity, the

TABLE 9

Proportion of wild type flies resulting from test of mating ability

	Proportion of wild type flies from cross A (a)	Proportion of wild type flies from cross B (b)
	0.7208	0.7414
	0.7558	0.7855
	0.7600	0.8230
	0.7754	0.8244
	0.7991	
Pooled estimate	0.7628	

mathematical model showing the effect of heterozygote-viability-modifying mutations may be developed.

MUKAI and BURDICK (1959) showed the following relationship in an equilibrium lethal heterozygote population:

$$f(\text{or } m) = \frac{2(1-Q)}{Q^2-4Q+2} \quad \text{.....(2)}$$

where f stands for the relative female fecundity and m for the relative male mating ability of lethal heterozygotes, assuming that the m's and f's of wild types are 1.00, and Q stands for the equilibrium frequency of the lethal heterozygotes.

The following expression can be obtained from Formula (1):

$$Q = 2 - (1/f)\ [1 \pm \sqrt{2(f-\tfrac{1}{2})^2+\tfrac{1}{2}}] \quad \text{.....(4)}$$

The negative root of formula (2) can be disregarded because Q should be between 0 and 1. Thus,

$$Q = 2 - (1/f)\ [1 + \sqrt{2(f-\tfrac{1}{2})^2+\tfrac{1}{2}}] \quad \text{.....(5)}$$

If we substitute R for $(1/f)\ [1 + \sqrt{2(f-\tfrac{1}{2})^2+\tfrac{1}{2}}]$, the zygotic array in the equilibrium population can be expressed as $(AA, Aa) = (R-1, 2-R)$, where "a" denotes the recessive lethal gene in question.

If a lethal-heterozygote-viability-modifying mutation takes place in the genetic background, it causes a change in the relative female fecundity from f to $(f+\Delta f)$. The frequency of individuals that have one or another of such new modifying mutations is c. This produces the following zygotic array:

$$(AA, {}_{\alpha}Aa, {}_{\beta}Aa) = [R-1,\ (2-R)\ (1-c),\ (2-R)c]$$

where α is the original genetic background and β the new genetic background carrying the mutation.

After one random mating generation, the frequency of the lethal heterozygotes would become:

$$Q' = \frac{R^2(2-R)\ (R-1) + (2-R)\ (R^2-2)\ \Delta fc}{R^2(R-1) + \Delta fc(2-R)\ (\tfrac{1}{2}R+1)\ (R^2-2)} \quad \text{.....(6)}$$

The increment of lethal heterozygote frequency (ΔQ) is expressed by the following formula:

$$\Delta Q = Q' - Q$$

$$= \frac{\Delta fc(2-R)\ (R^2-2)^2}{2[R^2\ (R-1) + \Delta fc(2-R)\ (R^2-2)\ (\tfrac{1}{2}R+1)]} \quad \text{.....(7)}$$

And, when $f > 1$,

$$(2-R) = \frac{2(f-1)}{[2f-1+\sqrt{2(f-\tfrac{1}{2})^2+\tfrac{1}{2}}]} > 0$$

$$(R^2-2) = \frac{2}{\sqrt{2(f-\tfrac{1}{2})^2+\tfrac{1}{2}}+(f-1)} > 0$$

Therefore, every term in Formula (7) is positive except Δf.

If we assume that an equal number of positive and negative mutations takes place, each having the same magnitude of effect, then the absolute values of the

numerator in Formula (7) are the same for a positive and a negative mutation. However, the denominator becomes smaller for a negative mutation and larger for a postive mutation making the positive effect on Q always less than the negative effect. Thus, a lethal gene has a destiny of elimination from the population by random spontaneous mutational changes of modifying genes even though the lethal heterozygote shows superviability associated with female fecundity (f). The same conclusion can be reached in the case of superviability associated with mating ability of males (m) because Formula (2) applies to both m and f.

Other factors which may have influenced the change in equilibrium frequency of lethal heterozygotes are: (1) Change in mating system. There was no intergeneration mating prior to the 16th generation in any of the four populations; there was intergeneration mating after that time. (2) Change in population size. The first 15 generations of each population began with 50 pairs of flies. The following generations began with more than 50 pairs. This would increase population size within the bottles and lead to greater competition between genotypes.

SUMMARY AND CONCLUSIONS

Various investigations concerning the lethal gene *l(2)55i* have been carried out in this study. Since this gene has previously been shown to exhibit heterosis (overdominance), an attempt has been made to determine how this heterotic effect is expressed.

1. Homozygous *l(2)55i* produces its lethal effect at some time during the larval period.

2. The gene *l(2)55i* was first found in, and extracted from, the wild type stock W–1 where the frequency of the heterozygote was about 0.34. The present test of lethal heterozygote frequency in W–1 yielded an estimate of 0.2353 ± 0.0420. This lethal has been maintained in this stock by random mating for more than four years.

3. Four experimental populations originally established in 1956 were tested for the present frequency of the lethal heterozygote. These populations differed from one another with regard to genetic background and initial lethal gene frequency. These four populations were found to have segregated from one another on the basis of genetic background. The two populations having W–1 genetic background were found to be fairly uniform in lethal gene frequency from bottle to bottle within each of the two populations, whereas the two populations having a mixed genetic background were found to have a variable lethal gene frequency from bottle to bottle within each of the two populations. Nevertheless, no bottle in any of the populations had lost nor nearly lost the lethal. The various estimates of the frequency of the lethal heterozygotes in the four populations were as follows:

Population 1, 0.1913 ± 0.0194; Population 2, seven estimates ranging from 0.1786 ± 0.0398 to 0.5078 ± 0.0578; Population 3, 0.2207 ± 0.0195; Population 4, ten estimates ranging from 0.1290 ± 0.0493 to 0.5714 ± 0.1063.

The estimates of lethal gene frequency in Populations 1 and 3 did not differ

significantly. A pooled estimate of lethal gene frequency in these two populations having W–1 genetic background is 0.2071 ± 0.0138. This pooled estimate is very near the lethal heterozygote frequency in W–1 (0.2353 ± 0.0420).

The equilibrium frequency of lethal heterozygotes was found to have changed from about 0.42 to about 0.20 over a period of 16 to 40 generations. This change can be attributed, at least in part, to random spontaneous mutational changes of modifying genes.

4. Experiments concerning the factors involved in the expression of heterosis were carried out using a homozygous wild type derivative of Population 1. Since this population has the W–1 genetic background, the results apply only to those populations with that background.

a. Zygotic viability was shown previously to be the same for lethal heterozygotes and the wild type. This fact was substantiated in this study.

b. Sperm competitive ability was shown to be the same for *l*-carrying and +-carrying sperm. This fact substantiates previous work.

c. The relative fecundity of $+/l$ females was measured giving two estimates of relative fecundity as follows:

1. 1.2102
2. 1.4030

The higher estimate of fecundity would be more than sufficient to support the equilibrium value of Q, 0.2071, while the lower estimate would require that the relative mating ability of $+/l$ males (when $+/+ = 1$) be 1.0910.

d. Experiments concerning the relative mating ability of $+/l$ males indicated that this value was not greater than unity.

The results of the above experiments indicate that the heterotic effect of the gene *l(2)55i* as expressed in those populations having the W–1 genetic background is due almost entirely to the greater fecundity of the lethal heterozygous females.

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