

THE EFFECT OF MATING STATUS, SEX AND GENOTYPE ON LONGEVITY IN *DROSOPHILA MELANOGASTER*^{1,2}

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LONGEVITY is an important component of fitness, particularly in organisms of short life span such as *Drosophila melanogaster* which normally produce eggs and sperm throughout their lives. All other factors being equal, individuals that live the longest produce the most progeny. Longevity is an expression of the entire organization of an individual under a sequence of environments. Both genetic and environmental aspects are involved; the latter may be either internal or external.

Some genes which have major effects on morphological traits may also influence life span (GONZALEZ 1923) but are not considered in this investigation. Longevity is treated as a metric trait with polygenic mode of inheritance.

Since 1910, numerous experiments designed to determine genetic and various gross environmental effects on longevity of *Drosophila* have been conducted. They have been reviewed by MALICK (1966). Few experiments investigated both genetic and environmental effects and the interaction between them. This investigation was designed to estimate genetic and environmental effects and their interaction on longevity of *D. melanogaster*. The genetic effects include heterosis, general and specific combining ability, maternal ability and residual reciprocal effects. The environmental effects include mating status and sex. Although genetically determined, sex effects result in gross physiological differences that may influence longevity. They are properly considered as environmental effects.

MATERIALS AND METHODS

A random mating reference population was constructed from four existing random mating wild-type stocks by making a 4×4 cross using ten males and ten virgin females of each stock. Five virgin females and five males were taken from each of the 16 resulting cultures and mass mated. At generation 15, an attempt was made to produce 20 isogenic lines by the marked-inversion-outcross technique described by KIDWELL (1963).

Five of the eight surviving isogenic lines were randomly chosen and a complete 5×5 diallel cross, including isogenic lines and reciprocals, was made. Each mating consisted of two males and three females.

An attempt was made to obtain 16 males and 16 females from each line and cross. Eight individuals of each sex were to be mated and eight maintained unmated as isolated individuals. Consequently, 100 "sex by mating status by genotype" subclasses were formed. Data on eight

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individuals were not obtained in each subclass. In some cases fewer than eight flies were available, and some escaped during transfer to new vials. Each subclass contained three to eight flies, the majority containing six to eight.

Flies were kept in shell vials with one experimental fly per vial. Mates were unrelated wild-type flies and were replaced if death occurred before that of the experimental fly, so that all mated experimental flies were with a mate until death. Vials were changed weekly before mold or cracks in the media developed. Flies were checked twice daily for deaths. Flies which became trapped in the media were released. This occurred more frequently toward the end of the life span. In all cases, a standard molasses-cornmeal media seeded with live yeast was used.

The average temperature during the experiment was 22.2°C with some fluctuation between 20° and 24°. The greatest extremes occurred during several days at the beginning of the experiment and were of short duration. Light and relative humidity were in the range normally encountered in an air conditioned laboratory, and were not measured.

The mathematical model used to describe the data is similar to the one described by KIDWELL (1963), but adapted to this experiment. The detailed model and analysis of variance are given by MALICK (1966) and are not described in detail here. The genetic elements in the model are assumed to be random and the environmental elements fixed. Consequently, inferences concerning the panmictic population which gave rise to the isogenic lines are possible.

Effects of general combining ability, specific combining ability, maternal ability and residual reciprocal effects and interactions involving them are measured among the line crosses only. The analytical procedures followed are those of GRIFFINGS (1956) "modified diallel" method 3. He has shown that it must be used to obtain unbiased estimates of the population parameters and make inferences concerning the random mating population from which the isogenic lines were formed.

RESULTS AND DISCUSSION

Preliminary analysis indicated that the variances within the smallest subclasses were unequal. A small nonsignificant correlation between means and variances of subclasses was found. A square root transformation did not remove the heterogeneity of variances, and all analyses were made on the original data. Tests of significance may be biased and are interpreted accordingly.

A least squares analysis as described by HARVEY (1960) was made using the complete model. Tests of significance and estimates of variance components were made in the usual manner. Many of the effects were not significant and the variance components small or even negative. These effects were deleted from the model, and a least squares analysis of the reduced model completed. A comparison of the two analyses indicates that although there are differences in the size of the variance components, the percent of the total variance contributed by each is not very different.

Means of the smallest subclasses are presented in Table 1, the analysis of variance of the reduced model in Table 2, and estimates of the variance components in Table 3. An approximate test of significance of mating status was made by addition of appropriate mean squares and the corresponding calculation of degrees of freedom as described by SNEDECOR (1956). Averaged over both sexes and breeding types, single flies lived about six days longer than mated ones. Similar effects of mating have been observed by DOANE (1960), RAMEL and EICHE (1960) and SMITH (1958a). These investigations, however, were not concerned with both sexes.

Linecross flies lived an average of 11.5 days longer than those from the isogenic

TABLE 1
Average longevity in days

Parental lines		Progeny			
Male	Female	Single		Mated	
		Male	Female	Male	Female
1	1	44.0	37.9	14.3	33.0
1	2	47.5	57.9	44.6	53.6
1	3	59.5	38.3	30.6	60.6
1	4	75.7	55.1	46.6	30.1
1	5	50.4	61.9	43.9	38.8
2	1	52.4	59.3	40.1	53.4
2	2	52.8	56.6	64.5	53.4
2	3	43.4	68.2	56.8	64.4
2	4	76.1	59.8	64.9	52.5
2	5	45.2	57.3	49.8	56.4
3	1	50.0	48.1	35.3	57.8
3	2	42.1	54.9	61.6	54.6
3	3	40.4	43.4	27.1	34.5
3	4	77.1	44.8	63.5	49.9
3	5	43.6	45.7	47.5	47.0
4	1	65.2	51.4	27.4	44.3
4	2	47.9	58.8	65.8	52.6
4	3	46.9	41.6	44.3	38.3
4	4	62.6	28.8	64.8	16.9
4	5	43.0	48.9	45.1	56.3
5	1	70.9	73.5	45.8	51.5
5	2	64.1	53.3	62.7	60.2
5	3	44.6	54.6	35.3	53.9
5	4	71.1	47.5	43.6	45.7
5	5	30.3	43.6	26.5	36.6

TABLE 2
Analysis of variance of longevity, reduced model

Source of variation		df	Mean square	Expected mean square
Description	Symbol			
Mating status†	T	1	4,545.5**	$\sigma_w^2 + 71.20\sigma_{tm;a2}^2 + 73.44\sigma_{tg;a2}^2 + 306.00K_t^2$
Breeding types	A	1	13,531.0**	$\sigma_w^2 + 210.40K_a^2$
Isogenic lines	D:A1	4	2,833.0**	$\sigma_w^2 + 24.48\sigma_{d;a1}^2$
Maternal ability	M:A2	4	1,256.3**	$\sigma_w^2 + 122.40\sigma_{m;a2}^2$
Specific combining ability	S:A2	5	544.5‡	$\sigma_w^2 + 48.96\sigma_{s;a2}^2$
Mating status × general combining ability	TG:A2	4	1,188.5**	$\sigma_w^2 + 73.44\sigma_{tg;a2}^2$
Mating status × maternal ability	TM:A2	4	644.6*	$\sigma_w^2 + 71.20\sigma_{tm;a2}^2$
Sex × isogenic line	BD:A1	4	3,465.8**	$\sigma_w^2 + 12.24\sigma_{bd;a1}^2$
Sex × maternal ability	BM:A2	4	2,009.2**	$\sigma_w^2 + 71.20\sigma_{bm;a2}^2$
Within	W	580	272.6	σ_w^2

† An approximate test of significance—see text.

‡ 10% level of significance. * 5% level of significance. ** 1% level of significance.

TABLE 3
Variance components

	Value	Percent
Among isogenic lines:		
Among isogenic lines, $\sigma_{d:a1}^2$	104.6	16.4
Sex \times isogenic line, $\sigma_{bd:a1}^2$	260.9	40.9
Within, σ_w^2	272.6	42.7
Total	638.1	100.0
Among linecrosses:		
Maternal ability, $\sigma_{m:a2}^2$	8.0	2.4
Specific combining ability, $\sigma_{s:u2}^2$	5.6	1.7
Mating status \times general combining ability, $\sigma_{tR:a2}^2$	12.5	3.8
Mating status \times maternal ability, $\sigma_{tm:a2}^2$	5.2	1.6
Sex \times maternal ability, $\sigma_{bm:a2}^2$	24.4	7.4
Within, σ_w^2	272.6	83.0
Total	328.3	99.9

lines, indicating a high degree of heterosis. Greater mean life span of hybrids has been observed by HYDE (1913), PEARL, PARKER and GONZALEZ (1923), CLARKE and SMITH (1955), and SMITH (1958b).

Differences among the isogenic lines were large and highly significant. Only average gene effects and their interactions are included in the genetic variances among isogenic lines ($\sigma_{d:a1}^2 = \sigma_A^2 + \sigma_{AA}^2 + \sigma_{AAA}^2 + \dots$ etc.). Since the numbers of each sex and mating status were nearly equal in each isogenic line, estimated interactions between additive effects of nonallelic loci contribute fully to response to selection among isogenic lines, but contribute only partly to selection response within a noninbred population. Therefore, heritability is estimated at 16.4% when selection is practiced among isogenic lines formed at random from the original population, but is expected to be lower when selection is practiced among individuals of the original panmictic population.

The general combining ability component of variance from linecrosses includes ($\frac{1}{2} \sigma_A^2 + \frac{1}{4} \sigma_{AA}^2 + \frac{1}{8} \sigma_{AAA}^2 + \dots +$ etc.), hence a comparison between the estimates of variance of additive effects from inbreds and hybrids can be made. General combining ability effects were small and nonsignificant indicating the absence of additive effects and interaction of additive effects among nonallelic loci. DICKERSON (1963) has pointed out that the effect of any gene difference and its contribution to genetic variance depends on the genetic and environmental background in which it is expressed. The average effect of the same gene difference may be quite different between isogenic line and linecross populations, as these results indicate.

The effect of sex averaged over single and mated flies of both breeding types was not significant. However, examination of Table 1 indicates large differences in some subclasses, and that the direction of the difference varies. MALICK (1966) has reviewed numerous experiments and found many inconsistent sex differences. She concluded that differences in strains, species, a wide variety of environmental

TABLE 4

Least squares sex × isogenic line, sex × maternal ability and mating status × general combining ability means

Subclass	Line				
	1	2	3	4	5
Sex × isogenic line:					
Males	27.88	57.33	32.53	62.44	27.17
Females	36.70	56.26	40.23	24.06	41.35
Sex × maternal ability:					
Males	43.22	47.96	41.33	60.49	40.45
Females	49.82	43.00	47.13	44.87	42.83
Mating status × general combining ability:					
Males	51.40	52.08	43.72	47.01	49.45
Females	35.76	51.02	45.84	40.51	39.43

factors and laboratory techniques contributed to the conflicting results. This suggests that interactions of sex with genetic and environmental effects may influence longevity. SMITH (1958a,c) found in *Drosophila subobscura* that mated males lived longer than mated females, but that longevity of females could be made greater than males by keeping them virgin or by exposing mated females to a high temperature for a short period, which caused partial regression of the ovaries. The heat treatment did not effect longevity of males. However, the sex by mating status interaction in the present study was small and nonsignificant.

A highly significant sex by isogenic line interaction resulted from a change in rank of lines between sexes (Table 4). Males in line 4, for example, had the longest, and females the shortest life span. This indicates an interaction of additive genetic effects with sex. The sex by general combining ability interaction, which contains corresponding estimates from the linecrosses, was very small and nonsignificant. The difference between these estimates further illustrates the possible differences in genetic parameters due to differences between homozygous and heterozygous genetic backgrounds.

A significant sex by maternal ability interaction was found, and is due to a change in line rank with respect to maternal ability between sexes (Table 4). A female's influence on her offspring is due both to her genotype and her environment. In this population, the different internal environments of sex may influence the expression of differences with respect to maternal ability. The activities of nurse cells are of primary importance in the formation of egg cytoplasm and yolk in developing oocytes of *D. melanogaster* (DOANE 1960). Endomitosis occurs in nurse cell nuclei (JACOB and SIRLIN 1959; PAINTER and REINDORP 1939) and may result in the breakdown of nuclear material. Possibly such breakdown products enter the oocyte along with other nurse cell contributions and interact differently with the genotype of the sperm or are utilized in such a manner that the internal environments of the sexes tend to reduce or enhance longevity.

Among linecrosses a small but highly significant effect of maternal ability was observed. These effects are not usually expected in *Drosophila*, but could be due

to causes similar to those discussed in connection with the sex by maternal ability interaction.

Although significant, the component attributable to specific combining ability is very small, amounting to only 1.7% of the total variance. It is concluded that dominance and epistatic effects contribute very little to the variance of longevity in this population.

The small but significant mating status by general combining ability interaction is due to a shift in rank with respect to general combining ability of single and mated flies (Table 4). These results support the general conclusion that in this population genetic sources of variation of longevity are very small.

SUMMARY

Five isogenic lines were used to make a complete diallel cross. An analysis was made of longevity including the effects of mating status, sex, heterosis, isogenic lines, general and specific combining ability, maternal ability, residual reciprocal effects and interactions among them. Mating status and sex were considered environmental factors.—Mating status, breeding type, and isogenic line had a significant effect on longevity. Single flies generally lived longer than mated ones, and hybrids lived longer than inbreds. Significant differences among isogenic lines were noted.—Significant sex by isogenic lines and sex by maternal ability interactions were observed. The effects of maternal ability and specific combining ability and the interaction of mating status with general combining ability and with maternal ability were significant.

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