NEGATIVE CORRELATION BETWEEN RATE OF DEVELOPMENT AND FEMALE FERTILITY IN *DROSOPHILA MELANOGASTER*^{1,2}

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THE fitness of an organism is the resultant of many components. Every character, even the most trivial, may contribute to the total fitness through one or more of the many biochemical pathways in the developmental process. These pathways are interrelated in an exceedingly complicated way. Yet the characters can be grouped into categories even if the interrelationships of the components within a category are not understood.

In this study rate of development (time from egg laying to eclosion) and fertility (number of progeny per adult female) were measured. The developmental rate is a major component of preadult fitness, and fertility the most important part of adult fitness.

BONNIER, JONSSON and RAMMEL (1959) have shown a high correlation between preadult viability, as measured by survival through the preadult stages, and the rate of development. Therefore a study of developmental rate also gives information about viability.

Preadult viability and female fertility have been studied in some detail in heterozygotes for second chromosome lethals in *D. melanogaster* (HIRAIZUMI and CROW 1960). Since the purpose of that work was simply to estimate the amount of inferiority of lethal heterozygotes for several fitness components, the data were not sufficient to make a detailed analysis of their interrelation. However it is interesting to note that in lethal heterozygotes these two components were positively correlated (although not statistically significant) while in the normal controls the correlation was significantly negative.

The purpose of the present study is to analyse this point as accurately as possible.

MATERIALS AND METHODS

The materials used in the present study came mainly from two sources.

1. Two sib-mated lines: (A) Madison Oregon-10 (abbreviated as M in the following). This line was isolated by the author from f Oregon stock (SEIGER 1958) but had no forked phenotype. Starting from a single male, chromosomes I, II, and III were made homozygous in the usual way by crossing to a multiple balancer stock (Basc; *SM1/Pm*; *Ubx*¹³⁰/*Sb*) to form the M Oregon-1

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line. Because of the simultaneous presence of inversions on three chromosomes there may have been some crossovers. However, after its construction this line was kept by sib-mating for ten generations before starting the present study. (B) 3V-30. This line was made homozygous for chromosomes I, II, and III by DR. SEIGER, starting from a single male which was captured at Vandeventer Flats. Since that time this line had been kept by sib-mating for 30 generations.

2. Selected lines for fast and slow rate of development: These were lines free of second chromosome lethals from the previous study (HIRAIZUMI and CROW 1960) which had been kept in our laboratory for about three years in small mass cultures. Twenty lines were chosen at random and mixed together, then kept for seven generations in one large mass culture without any conscious selection. Nineteen pairs were then chosen for the selection experiment.

Among the 19 culture vials two, one showing the fastest and one showing the slowest average developmental rate, were chosen to start the fast and slow lines. About ten pairs which eclosed on the very first day were then chosen from the F_1 's of the fastest culture. This process was repeated twice, inserting two generations of mass transfer without selection between the selected generations. Therefore there was a total of three generations of selection for the fast line. Since, as will be seen later, heterozygotes generally show faster development than homozygotes, there may have been some selection for heterozygosity. A similar selection procedure was followed for the slow line, except that it continued for five generations. Since the fertilities of the pairs eclosed at the end of a culture were generally very low (often they were completely sterile), pairs to continue the selection were chosen from flies eclosed one or two days before the last ones emerged. Therefore the selection intensity might be slightly weaker for the slow line than for the fast.

Starting from these selected lines and using the multiple balancer stock, one fast and two slow developmental rate lines homozygous for the second and third chromosomes were established (19A for the fast line, 12A and 12B for the slow lines). Mating schemes were designed to replace the sex chromosomes by those of the M line. The fourth chromosome was entirely ignored in this study on the assumption that its contribution, if any, would be very small.

Since a slight change in environment may cause a substantial change in the rate of development, a trial to get relatively comparable culture conditions was made in the following way: One (sometimes more) fertilized female(s) of the genotype in question and one fertilized *cn bw* female were put together into a food vial and allowed to lay eggs for 24 hours. Males were, of course, discarded before the tests to avoid undesired matings.

Counts were made twice a day until all the flies had eclosed. The average time from egg laying to eclosion was then computed for both genotypes separately in the two sexes, and only those cultures in which the unweighted average eclosion time of the *cn bw* males and females lay between 9.00 and 10.50 days were selected for the analysis.

In order to minimize the effects of environmental differences, the following

analysis was used. Let x and y be the eclosion time of the *cn bw* strain and of the genotype in question, taking the unweighted average for the two sexes. Since the value of x was variable within the chosen interval 9.0 to 10.5, it is necessary to adjust the y value to some constant x within this range. For this purpose the linear regression of y on x was computed for each genotype, and the average developmental rate of y was estimated at the point $x = 9.80$. The averages of x for almost all genotype sets were close to this value, and this was chosen because the standard error of the estimate of y is the smallest at the average of x .

Female fertility was measured as follows: One female of age less than 12 hours was put into a food vial together with three Canton-S males between one and two days old. Every three days they were transferred to a fresh food vial for three changes. Therefore each female was allowed to lay eggs for nine days, and then the adult progeny were counted. The tests were replicated at several different times. At each time the M line was included in each group to check the effect of possible secular environmental fluctuations but since there was no, or very little, environmental effect, all the data were treated as if they were obtained at the same time.

Maternal effect: Several crosses were made reciprocally to detect a possible maternal effect on the rate of development and female fertility. The results are summarized in Table 1. In all but one (cross No. 5 in fertility) there was no significant difference between the reciprocal crosses. In cross No. 5 the difference was significant but a true maternal effect is quite unlikely in this case because the F_1 females were less fertile when they were the descendants of the more fertile mother. This difference might be due either (1) to a somehow poor culture condition from which the tested F_1 females were collected or (2) merely to chance which might produce apparently significant results among so many tests.

Thus there is so far no demonstrable maternal effect when each set of reciprocal crosses is examined separately, but still there may be a significant difference when all the data are pooled. The sum of the eclosion times of the F_1 genotypes whose mothers showed a faster rate is 46.01 ± 0.14 compared with 46.10 ± 0.15 for the reciprocal crosses which is not significant difference. In fertility, the total of F_1 females whose mother showed higher fertility is 3625 ± 50 while that of the reciprocal cross is 3691 ± 55 , which also is not significantly different. Therefore we conclude that there is extremely little, if any, maternal effect.

Interaction between chromosome II and III: For further analysis it is necessary to know how the individual chromosome pairs contribute to a fitness component. To answer this question we first seek an approximate but general rule which the majority of chromosome pairs obey, then proceed, if necessary, with various modifications, to improve the accuracy of the approximation. This can be done by examining the amount and direction of deviations of the observed results from the values which are expected based on the first approximation rule.

There are two possible simple rules, (1) complete additivity and (2) completely multiplicative contribution from each chromosome pair. When the dif-

TABLE 1

Time of eclosion and female fertility in reciprocal crosses. M, 3V, and 12B are chromosomes described in the text. pI is P₁-I Oregon (SEIGER 1958)

Cross no.	Parents Chromosome constitution						Offsprings		
	Female			Male			Female		Male
	I	II	III	I	II	III	Time of eclosion	Fertility	Time of eclosion
1	M	M	M	3V	3V	3V	9.09±0.09	545±12	
	3V	3V	3V	M	M	M	9.09±0.04	568±13	
2	M	M	M	pI	pI	pI	9.67±0.09	364±25	
	pI	pI	pI	M	M	M	9.59±0.05	420±19	
3	pI	pI	pI	3V	3V	3V	8.96±0.06	531±19	
	3V	3V	3V	pI	pI	pI	9.02±0.09	526±20	
4	M	M	M	M	M	3V	9.10±0.03	509± 9	9.28±0.04
	M	M	3V	M	M	M	9.06±0.06	484±10	9.25±0.05
5	M	M	M	M	3V	3V		498±14**	
	M	3V	3V	M	M	M		581± 5	
6	M	M	M	M	12B	M		374±12	
	M	12B	M	M	M	M		314±38	
7	M	M	M	M	M	12B		468±17	
	M	M	12B	M	M	M		455± 5	
8	M	M	M	M	12B	12B		336±25	
	M	12B	12B	M	M	M		343±23	

** This difference is significant at the one percent level.

ferences among the chromosome in question are very small these two rules give approximately the same expectations, but if the differences are large the first sometimes gives biologically meaningless expectations, such as negative fertility, while the second always remains meaningful. As a matter of fact, as will be seen later, the chromosome pairs obey the multiplication rule somewhat closer than the addition rule, and hence this is the candidate for the first approximation rule. For a statistical test of goodness in fit, it is more efficient to transform the observed values into logarithms, which converts the multiplication into addition. Normality tests were applied to the distributions with the two scales, but neither deviated significantly from the normal distribution. However, since the logarithmic transformation appeared to make the distribution somewhat less normal in the female fertility distribution, and since the observed values are easier to understand biologically, only the original untransformed values are presented

TABLE 2
Time of eclosion and female fertility in various combinations of chromosome II and III

Designation	Homozygote				Heterozygote			
	Chromosome II	Chromosome III	Time of eclosion	Female fertility	Chromosome II	Chromosome III	Time of eclosion	Female fertility
A	M/M	M/M	9.64±0.06	397±12	M/M	M/M	9.64±0.06	397±12
B	19A/19A	M/M	9.42±0.04	308±18	19A/M	M/M	9.28±0.09	462±11
C	M/M	19A/19A	9.50±0.07	280±16	M/M	19A/M	9.44±0.05	460±4
D	19A/19A	19A/19A	9.31±0.05	220±24	19A/M	19A/M	9.01±0.05	497±15
	A × D		89.75±0.70	87.3±9.8			85.86±0.67	197.3±8.9
		B × C	89.49±0.75	86.2±7.2			87.60±0.99	212.5±5.4
B	3V/3V	M/M	9.39±0.12	312±21	3V/M	M/M	9.03±0.06	430±24
C	M/M	3V/3V	9.48±0.04	261±16	M/M	3V/M	9.18±0.04	496±7
D	3V/3V	3V/3V	9.05±0.08	169±24	3V/M	3V/M	9.05±0.06	542±7
	A × D		87.24±0.95	67.1±9.9			**87.24±0.76	215.2±6.7
		B × C	89.02±1.17	81.4±7.3			82.90±0.64	213.3±12.4
B	12A/12A	M/M	9.83±0.09	162±17	12A/M	M/M	9.05±0.14	361±29
C	M/M	12A/12A	10.13±0.12	74±13	M/M	12A/M	9.37±0.04	462±13
D	12A/12A	12A/12A	10.10±0.35	41±7	12A/M	12A/M	9.12±0.16	457±17
	A × D		97.36±3.42	16.3±2.8			87.92±1.63	181.4±8.7
		B × C	99.58±1.51	12.0±2.5			84.80±1.33	166.8±13.3
B	12B/12B	M/M	9.32±0.04	168±32	12B/M	M/M	8.95±0.13	346±19
C	M/M	12B/12B	10.05±0.21	246±29	M/M	12B/M	9.09±0.05	461±8
D	12B/12B	12B/12B	9.60±0.08	144±17	12B/M	12B/M	8.61±0.13	340±17
	A × D		92.54±0.94	57.2±6.9			83.00±0.63	*135.0±8.0
		B × C	93.67±2.02	41.3±9.4			81.36±1.23	159.5±9.1

* Significant at the five percent level.
** Significant at the one percent level.

in Table 2. In fact, the conclusions are the same whether based on transformed or raw data.

Let A and D be the measures for the two original combinations of chromosome II and III, B and C be the measures for the two genotypes obtained by exchanging the two chromosome pairs reciprocally. Then under the rule of multiplicative action, $BC - AD = 0$. In two cases this value was significantly different from zero but since there were 16 cases examined (eight cases for developmental rate, eight for fertility) this deviation might well be expected merely by chance alone.

There was, however, an apparent over-all tendency. With low values of BC , AD tends to be greater than BC . This was tested by computing the correlation between BC and $(BC - AD)/BC$. The correlation was positive and significant for developmental rate ($P < 0.05$) but not for female fertility.

Although there is therefore some evidence for interaction of chromosomes, this may be due to metrical bias. In any event the amount of interaction is small and may be neglected in the further analysis without serious error.

Relation between developmental rate and female fertility: We shall proceed as if there is no interchromosome interaction. Then we can estimate the contribution of each tested chromosome from two independent observations. For simplicity, the values of the chromosomes II and III from all crosses were measured relative to the M line. The relative level of the tested chromosomes II and III was measured by taking the square root of BD/AC and CD/AB respectively. The estimates for eclosion time thus obtained were always smaller in chromosome II's (i.e., development was faster) than that in the chromosome III's. This suggests that the chromosome II of strain M contributes more to slow development than its chromosome III does. Since the average of this difference was about 0.010, we assign the value 1.005 and 0.995 to the chromosomes II and III of strain M respectively. The contribution to the relative developmental time of each tested chromosome was re-computed on the basis of these values. No correction was made for fertility because there was no such clear difference between chromosome II and III. We therefore assign the value 1.00 for both chromosomes II and III of M line. After making these corrections we obtain the results given in Table 3.

When the developmental rate of the chromosome is faster (i.e., eclosion time less) than the level of 1.000 the correlation between the time of eclosion and female fertility is significantly positive ($r = +0.92$, $n = 4$, $P \leq 0.01$). Since the larger value in the time of eclosion means the slower rate of development, positive correlation means that the developmental rate is negatively correlated with female fertility. But when development is slow the correlation becomes negative though not significant ($r = -0.45$, $n = 2$, $P > 0.05$ for the four items at the bottom of Table 3).

The former relation is also true in the heterozygous condition, ($r = +0.800$, $n = 6$, $0.02 < P < 0.01$).

Thus the negative correlation between female fertility and developmental rate when the latter is fast is now established.

TABLE 3

Relation between time of eclosion and female fertility for the contribution of various chromosomes relative to the M chromosome

Homozygous condition			Heterozygous condition with M		
Chromosome	Time of eclosion	Female fertility	Chromosome	Time of eclosion	Female fertility
M (II)	0.995	1.00			
19A (II)	0.984	0.78	19A (II)/M (II)	0.963	1.12
19A (III)	0.982	0.71	19A (III)/M (III)	0.970	1.12
3V (II)	0.969	0.71	3V (II)/M (II)	0.966	1.09
3V (III)	0.969	0.60	3V (III)/M (III)	0.972	1.25
12B (II)	0.966	0.50	12B (II)/M (II)	0.943	0.80
M (II)	1.005	1.00			
12A (II)	1.013	0.48	12A (II)/M (II)	0.961	0.95
12B (III)	1.031	0.73	12B (III)/M (III)	0.947	1.07
12A (III)	1.034	0.22	12A (III)/M (III)	0.984	1.22

DISCUSSION

The data obtained earlier for another study (HIRAIZUMI and CROW 1960) can also be used to measure the relation between developmental rate and fertility. The correlation coefficient for normal second chromosomes in the heterozygous condition is +0.600 ($P=0.02$), in agreement with the present results. Thus in two independent studies in highly viable populations a negative correlation between two principal components of fitness is shown to exist. This has been expected on theoretical grounds (ROBERTSON 1955) and has been discussed by several writers (see for example, FALCONER 1960).

On the other hand the correlation becomes positive when less fit genotypes are studied. One line of evidence comes from the fact that lethal heterozygotes as a group are low in both fertility and rate of development (HIRAIZUMI and CROW 1960). This same study showed a positive though not significant correlation ($r=-0.225$) between fertility and developmental rate of individual genotypes, each heterozygous for a different lethal.

The data in the present study are in agreement, though the numbers are too small for statistical significance. They do show, when taken at face value, a positive correlation between the two components when both are at a low level.

Natural selection favouring intermediate phenotypes has been discussed theoretically in various places but no consistent experimental evidence has been given on how it operates. The present study may give a hint for this question. The two major components are negatively correlated within a certain range of values. In natural populations the maximum fitness combinations of these two components must be located somewhere in this high range. A reduction in one character involves an increase in another. Thus they somehow compensate in a certain range but beyond it an increase in one component may become insufficient to compensate for a reduction in the other resulting in a decline in the total fitness.

Hence the more fit genotypes must have an intermediate level in both components.

Assuming a linear relationship between the two components and independent contribution of chromosome pairs (these assumptions may be approximately true within a relatively small range of their levels) the combinations of two chromosomes which show similar levels in developmental rate give higher fertility, hence the combinations of similar levels must generally have a selective advantage and will be accumulated in a population.

In natural populations of *Drosophila* the generation times overlap, and this is especially important during the warm season when the population size increases rapidly. For such a species fitness is best estimated in terms of FISHER's Malthusian parameter, m , (FISHER 1930) which is defined as a real root of the following equation.

$$\int_0^{\infty} l_x b_x e^{-mx} dx = 1$$

Here l_x is the proportion of survivors to the age x , b_x is the average number of offspring per individual during the age interval dx . In *Drosophila* l_x is measured by the proportion of fertilized eggs which survive to the age x . Since the proportion of survivors after eclosion remains nearly 100 percent for the most important first two or three weeks (HIRAIZUMI and CROW 1960) l_x may be taken as unity through this period (this excludes the larval stage). The number of female progeny per parental female at age x gives a measure of b_x . The lower limit of integration is chosen as the age when a genotype in question reaches adulthood. A good measure for this is the time from egg laying till eclosion.

It is of interest to compare the contribution of fertility and rate of development in determining total fitness. In *Drosophila* cultures the death rate is low during early adult life, and the egg laying is roughly constant during the first two or three weeks. Therefore we take $b_x = c$ for the interval $14 \leq x \leq 33$ days. Thirty-three days is taken arbitrarily because fertility was measured over this range in the earlier experiments. However, the upper limit of the integration is of little consequence in determining the measure of fitness. We also take $l_x = (1-s)$ to include the larval stage mortality, where s is a measure for the death rate at this stage.

Then the Malthusian parameter, m , is given by

$$c(1-s) \int_{14}^{33} e^{-mx} dx = 1$$

or roughly

$$c(1-s)e^{-14m} = m$$

A simple example will give us an understanding of how much the two factors contribute to the total fitness.

Let us consider four model cases.

		$c(1-s)$	K
Case 1	a	5.00	11.4
	b	5.25	12.0
Case 2	a	5.00	13.3
	b	5.25	14.0
Case 3	a	5.00	15.2
	b	5.25	16.0
Case 4	a	2.50	15.2
	b	2.625	16.0

where $c(1-s)$ is the survival and fertility factor and K is the mean days from egg to adulthood. In all four cases K and $c(1-s)$ are smaller (i.e., faster development but less fertile) in *a* than in *b* by five percent. The m value for each case was computed approximately, and the values are listed in the following table.

		m	c'	$(c'-c)/c \times 100$
Case 1	a	0.2595		
	b	0.2536	5.84	16.8%
Case 2	a	0.2311		
	b	0.2247	5.87	17.4%
Case 3	a	0.2092		
	b	0.2027	5.95	19.0%
Case 4	a	0.1748		
	b	0.1703	2.87	14.8%

where c' is the value of $c(1-s)$ in *b* to give the same m value as in *a* when K remains the same, and $(c'-c)/c \times 100$ is the percent increase in $c(1-s)$ to com-

pensate for a five percent reduction in the developmental rate. It is clear from the table that it is necessary to increase the fertility factor by 15 to 19 percent to overcome a five percent reduction in the developmental rate. This required percent increase becomes larger as K gets larger with the same fertility (cases 1 to 3). In other words when the life cycle of the flies goes slowly, the developmental rate becomes more important relative to the fertility. The comparison between cases 3 and 4 shows that when the fertility factor is small, fertility is more important relative to the developmental rate. All these relations may be much more complicated in natural populations, but in an organism like *Drosophila* with early maturity and long reproductive time the developmental rate is an important part of the total fitness.

SUMMARY

The relation between the rate of development and female fertility was studied for chromosomes II and III of *D. melanogaster*. The principal conclusions were as follows:

1. There was no detectable maternal effect in the two components.
2. To a first approximation chromosome II and III contributed to each component in a simple multiplicative fashion although significant, but small, deviations from this rule were observed.
3. The rate of development was negatively correlated with female fertility when the developmental rate was faster than a certain level but positively when it was slower than this level.
4. Some possible meanings of this negative correlation in natural populations, and the relative importance of developmental rate in determining the total fitness were discussed.

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