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Maternal effects on the rate of egg development in *Drosophila subobscura*

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1. INTRODUCTION

It was shown by Maynard Smith & Maynard Smith (1954) that outbred *Drosophila subobscura* develop more rapidly than inbred ones, as judged by the time from egg-laying to the emergence of adults, and that they are less variable at a given temperature. It was possible in these experiments to eliminate maternal and environmental effects by comparing two classes of offspring, respectively homozygous and heterozygous for genes on chromosome 5, of the same parents, and to show that more rapid development can be caused by genetic heterozygosity.

The purpose of the present investigation was to discover whether there are genetically determined differences between the rates of development of eggs, as judged by the time from laying to the hatching of larvae, and, if there are such differences, whether they depend on the genotype of the eggs themselves, or of the females which lay the eggs.

2. METHODS

Eggs from four inbred lines, *NFS*, *K*, *B* and *M*, were studied, and also F_1 eggs, and eggs obtained by crossing F_1 hybrids to unrelated inbred lines and to unrelated F_1 hybrids. The *NFS*, *K* and *B* lines are structurally homozygous for all chromosomes, and had been brother-sister mated for 50, 64 and 65 generations respectively. The *M* line had been brother-sister mated for 25 generations, but was not structurally homozygous. F_1 hybrids are written, for example, *M*/*NFS*, the female parent being written first.

The following kinds of paired mating were set up, as indicated by crosses:

	Male					
Female	*NFS*	*K*	*B*	*M*	*B*/*K*	*M*/*NFS*
NFS	×			×	×	
K		×	×			×
B		×	×			×
M	×			×	×	
B/*K*	×			×		×
M/*NFS*		×	×		×	

Forty to fifty pairs each were set up of matings involving inbred females, and fifteen to twenty pairs each of matings involving outbred females. Eggs were col-

lected from these females, 7 to 11 days after emergence, on a dark medium consisting of agar and molasses with living yeast suspension added, the medium being poured onto slips of balsa wood which fitted tightly into 3-in. by 1-in. dia. vials.

It was desired to know within one hour the time at which the eggs had been laid, and also, in order to eliminate differences in hatching time due to small temperature fluctuations, to collect eggs from all matings simultaneously, as far as this was possible. Accordingly the males were discarded after an adequate period had been allowed for mating, and eggs collected from the females for eight successive hourly periods, at the end of which time a sufficient number of eggs had been obtained from most types of mating. Unfortunately, the *K* females laid relatively few eggs, as did the *B* females mated to *M*/*NFS* males.

A complication in the procedure was imposed by the necessity of avoiding 'stored' eggs. A female can retain one fertilized egg in the vagina for many hours before laying it; such eggs hatch in a shorter time than do unstored eggs. Therefore, at the start of the experiment all the females were transferred individually onto dark medium, which was then examined at approximately hourly intervals. No female was used for the experiment proper until she had laid at least one egg. As soon as an egg was seen, the female was grouped together with a few others of the same kind, and transferred to fresh medium at hourly intervals. Only the eggs laid on these later drops of medium were recorded.

In this way some hundreds of vials were obtained containing medium on which eggs of known genotypes had been laid during known periods of one hour. Since it was desired to examine the eggs hourly for hatching, the number of vials to be examined had to be reduced. Therefore all eggs from the same kind of mating laid during the same hour were transferred to a single drop of medium, and arranged in rows to facilitate counting. Since there were still too many vials, forty-five such 'batches' of eggs were selected for actual recording of hatching time, the selection being made to give as far as possible a reasonable sample from all types of mating.

These forty-five batches of eggs were examined hourly, and the number of unhatched eggs recorded. Counting was started 36 hours after the first eggs were laid, and continued until 59 hours after the last egg was laid, at which time, since no further eggs had hatched during the last hour, it was judged safe to stop. A check of the unhatched eggs 24 hours later showed that four further eggs had hatched; these have been ignored below, but had they been included it would not have altered any of the conclusions drawn.

The vials containing eggs, except when being examined, were kept covered with a damp cloth, beneath which the temperature was between 16·5° and 17°C.

3. RESULTS

Table 1 gives for each type of mating the number of eggs hatching as a fraction of the total, and the mean hatching time in hours. The last two columns of the table give the mean and standard deviation of the hatching time for all the eggs laid by the six kinds of female.

An analysis of the results is given in Table 2, which shows that the major part of

the variation in hatching time was due to differences in the development rate of eggs laid by different kinds of female. The slowest eggs were laid by *NFS* females; this inbred line was derived by Hollingsworth & Maynard Smith (1955) from a wild-caught female by combining inbreeding with selection for slow development.

Table 1. *Fraction of eggs hatching, and mean hatching time in hours, for various types of mating*

	Male						All males	
Female	*NFS*	*K*	*B*	*M*	*B/K*	*M/NFS*	Mean	Standard deviation
NFS	30/57 54·37			33/63 50·39	27/29 50·93		51·88	1·69
K		16/33 51·19	5/9 49·00			7/10 49·71	50·43	1·52
B		39/55 50·00	28/33 49·10			6/7 50·83	49·59	1·25
M	43/59 46·61			26/56 47·42	43/50 48·21		47·41	1·28
B/K	33/33 48·06			17/36 46·76		55/56 48·07	47·86	0·94
M/NFS		34/46 47·59	27/30 46·52		68/68 46·03		46·54	1·13

Although selection was relaxed after seven generations, *NFS* flies still take 3 to 4 days longer to emerge than flies from other inbred lines. Eggs laid by *B* and *K* females were intermediate in development rate, and the fastest eggs were laid by the F_1 hybrid females *B/K* and *M/NFS*, and by inbred *M* females.

The effects on hatching time of the zygotic nucleus can be estimated by comparing eggs laid by females of a given type mated to different kinds of males. These effects are much smaller than the maternal effects. Before deciding whether they are statistically significant, an estimate of the 'error variance' must be made.

Table 2. *Analysis of variance of egg hatching time*

	Sum of squares	Degrees of freedom	Variance	*P*
Within batches	794·1	492	1·614	
Between batches of the same genotype	120·2	27	4·45	< 0·001*
Total within genotypes	914·3	519		
Between males { with *NFS* females	283·0	2	141·5	< 0·001†
Between males { with other females	178·9	10	17·9	< 0·01†
Total within females	1376·2	531		
Between females	1888·0	5	377·6	
Total	3264·2	536		

* Comparison of variances within and between batches.
† Comparison with the between-batch variance.

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The first two rows of Table 2 compare the within-batch variance, due to differences between eggs laid by females from the same type of mating in the same hour, with the between-batch variance, due to differences between eggs laid by females from

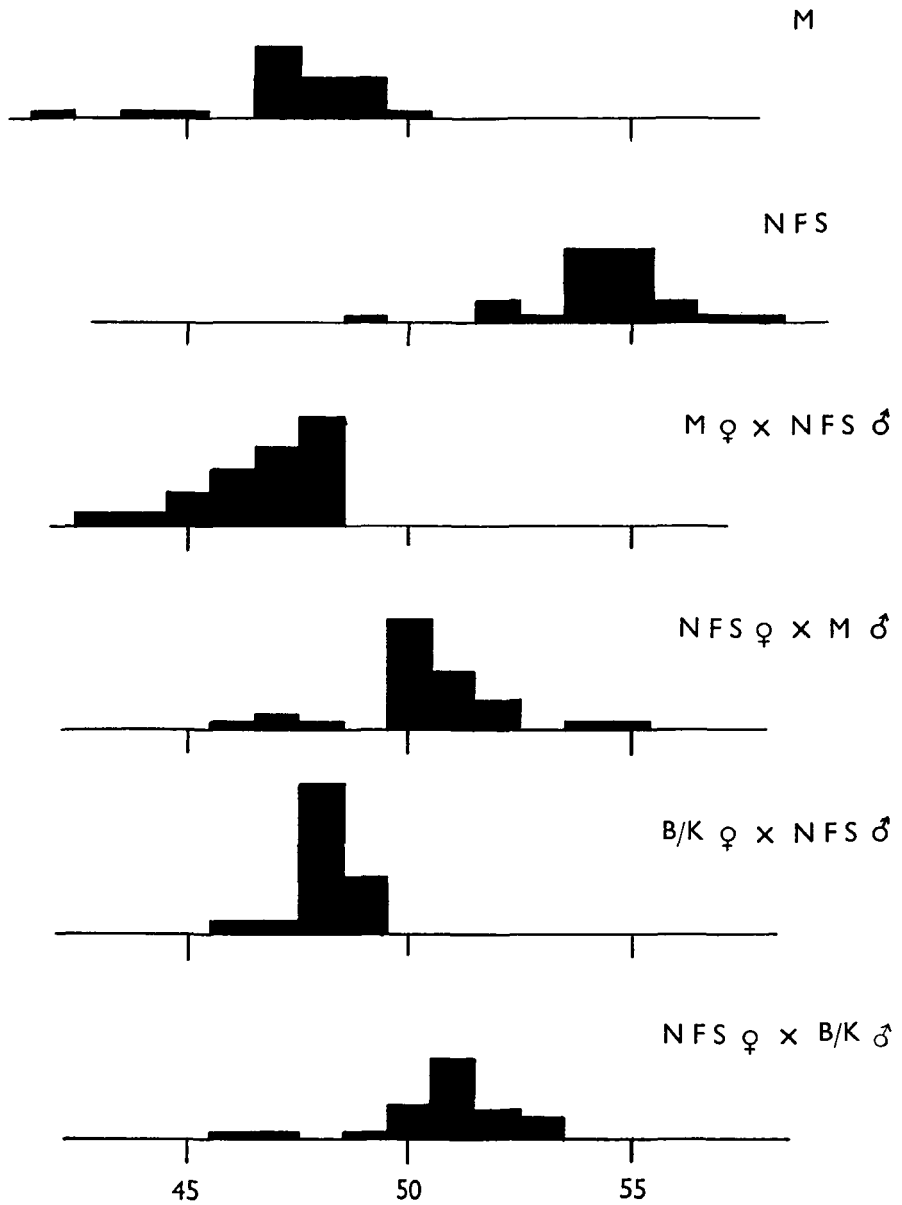

Fig. 1. Hatching times in hours at 17° C.

the same type of mating in different hourly periods. The latter variance, although it contributes little to the total, is significantly greater than the within-batch variance. Presumably this reflects slight differences in the temperature or humidity

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to which different batches were exposed. Therefore, in estimating the significance of nuclear effects, these must be compared with the between-batch variance.

Differences due to the zygotic nucleus have been analysed into two parts. First, there is a highly significant contribution to the total variance due to the fact that eggs laid by *NFS* females develop at different rates according to the male which fertilizes them. Table 1 shows that *NFS* eggs fertilized by *NFS* sperm took about 4 hours longer to hatch than did *NFS* eggs fertilized by sperm from an unrelated male; it does not matter whether the unrelated male is inbred or outbred.

The remaining effects of the zygotic nucleus, for eggs laid by females other than *NFS*, are just significant at the 0·01 probability level, but it is far from clear what they signify. As Table 1 shows, it is not true that eggs laid by inbred females (other than *NFS*) develop more slowly if fertilized by sperm from the same inbred line, nor is it true that eggs develop more slowly if fertilized by *NFS* sperm.

Finally, the data give some information on the variability of development rate. The standard deviations given in the last column of Table 1 are the square roots of the within-batch variances for each kind of female; the values would have been slightly higher had the between-batch variances been included. It will be seen that eggs laid by F_1 hybrid females are less variable than the eggs laid by inbred females.

The various conclusions are illustrated in Fig. 1, a selection of the data which shows the differences between reciprocal hybrids due to the maternal effects, and also the slow development and greater variability of eggs laid by *NFS* females, particularly when fertilized by *NFS* sperm.

4. CONCLUSIONS

The rate of development of *Drosophila* eggs, from laying to hatching, is in the main determined by the female laying the eggs, and only to a small extent by the genotype of the embryo. This agrees with the finding of Moore (1933), working with species hybrids in echinoderms, that cleavage rate is determined by the cytoplasm and not by the nucleus. Later, in the larval and pupal development of *Drosophila*, the work of Maynard Smith & Maynard Smith (1954), and other unpublished data, suggest that the nuclear genotype of the individual is of major importance in determining development rate. But this may not always be the case; Clark (1957) found that there was a large difference in development rate between the species *D. setifemur* and *D. spinofemora*, and that F_1 hybrids between these species resembled their mothers in the time taken to develop from egg to adult.

One case only was found in which the male parent had a large effect on the rate of egg development. Eggs laid by inbred *NFS* females develop more rapidly if fertilized by unrelated males. In this case it seems that the sperm can in part compensate for some deficiency of the egg cytoplasm, probably through the chromosomes which it contributes to the zygotic nucleus.

Although the rate of egg development is in the main determined maternally, and of later development by the genotype of the developing individual, it is interesting that those genotypes which cause individuals to develop rapidly also cause those individuals, if female, to lay eggs which develop rapidly. Thus F_1 hybrids between

inbred lines develop into adults more rapidly, and with less variation of development time, than do inbred individuals, and F_1 hybrid females also lay eggs which develop more rapidly and are more uniform than the eggs laid by inbred females. Similarly, *NFS* individuals take longer than other inbred individuals to develop into adults, and *NFS* females lay eggs which take longer to hatch.

SUMMARY

The time in hours from laying to hatching at 17°C. has been measured for eggs from four inbred lines of *Drosophila subobscura*, and also for F_1 eggs, and for eggs obtained by crossing F_1 hybrids to unrelated inbred lines and to unrelated F_1 hybrids.

Eggs laid by females of a given genotype have a characteristic rate of development, which, with one exception, is not greatly influenced by the genotype of the male parent. F_1 hybrid females laid eggs which were more uniform in hatching time than eggs laid by inbred females, and which developed more rapidly than eggs laid by three of the four kinds of inbred female studied. The slowest-developing eggs were laid by females from the *NFS* inbred line, a line originally selected for slow development from egg to adult.

The male parent, and hence the zygotic nucleus, had an appreciable effect on the rate of egg development only for eggs laid by *NFS* females; these eggs developed more rapidly if fertilized by sperm from an unrelated male.

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