IMPORTANCE OF THE COMPOSITION OF THE DIET TO THE MANIFESTATION OF SIGNS OF FORKED MUTATIONS IN THE OFFSPRING OF *Drosophila melanogaster*

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We have previously reported certain embryological and medical grounds for the study of the action of the environment on the development of inherited signs in ontogenesis [4]. We have also reported experimental data relating to the action of the temperature and the character of the diet given to larvae and the parent generation of forked mutants of Drosophila melanogaster, on the mean number of atypical chaetae (macrochaetae) in their offspring [5]. A critical period in the development of macrochaetae was found at the end of the larval period, when a change of temperature could lead to a sharp increase in the degree of expression of the signs of mutation in the developing flies.

It has also been found that the number of anomalous chaetae in the offspring of parents receiving a raisin diet is lower than in the offspring of flies receiving a yeast diet (for the composition of the diets, see below). In females developing from the eggs of flies cultivated on a yeast diet, the mean number of anomalous chaetae was 7.59 ± 0.19, and on a raisin diet 4.5 ± 0.16. Such a considerable approximation of an inherited sign to normal could be attained by changing the diet of the parents only: feeding larvae of "yeast-fed" parents with a raisin diet caused hardly any decrease in the number of anomalous chaetae in the flies developing from them. Hence it followed that, besides some influence during the critical period at the end of the larval and beginning of the pupal period, the influence of the environment on the gametes of the parent generation is of importance to the formation of the chaetae.

In order to understand this problem correctly, it was necessary in the first place to determine the relative importance of the male and female gametes in this particular change in the degree of expression of the signs of mutation under the influence of a change in the diet. As test object we used the forked mutation of Drosophila melanogaster, as in our previous experiments [5]. This mutation is characterized by the fact that some of the large sensory chaetae on the surface of the body (macrochaetae) are abnormal: they may be greatly thickened, curved, hook-shaped, duplicated, and so on. We compared the mean number of anomalous macrochaetae on the whole body of flies, the parents of which were kept and reared in the larval state, like several of the preceding generations, on a raisin or a yeast diet. Separate counts were made of the macrochaetae on the tergites of the thorax, the head, and the pleurites. The yeast diet was prepared according to the formula: water 100 ml, agar 1.5 g, fresh yeast 15 g, semolina 5 g; the raisin diet consisted of 200 ml water, 1 g agar, 100 g boiled potato, and 100 g crushed raisins. As they emerged from the pupae, the males and females were quickly placed in separate tubes and fed on one of the two diets. At the age of 2 days the males and females receiving different diets were paired in the following combinations: 1) yeast ♀ × yeast ♂; 2) yeast ♀ × raisin ♂; 3) raisin ♀ × yeast ♂; and 4) raisin ♀ × raisin ♂. After 2 days the tubes were released, and the mean number of atypical chaetae was determined in the generations of flies developing from the eggs deposited on the food during this time. Counts were made only in females, for they had more atypical macrochaetae than the males (the "forked" gene is carried on the X-chromosome). The chaetae on 100-200 females in each experimental group were counted. The experiments were repeated several times.

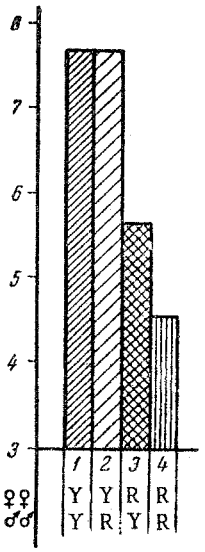
It will be clear from the table and figure that the largest deviation from normal development was found, as in previously published work [2], when both parents were fed on a yeast diet. When "yeast" females were crossed with "raisin" males the result was unchanged, but on the other hand, crossing "raisin" females with "yeast" males led to a marked decrease in the number of anomalous chaetae (the difference between the mean values was more than six times the possible error). The impression was gained that the approximation of the signs of the offspring to normal was influenced only by a change in the feeding of the females, and that the male gametes played no part in this phenomenon. However, if the figures of the fourth group of experiments were taken into consideration, it became

clear that this impression was wrong: if the "yeast" males were replaced by "raisin" males, the inherited anomaly of development of the latter was lowered still further, by a statistically significant amount. When we assessed the relative importance to the offspring of the changes, caused by the diet, in male and female gametes, we came to the conclusion that the transfer of both mothers and fathers from a yeast to a raisin diet had a normalizing influence on the offspring. However, the conditions of feeding of the female gametes were incomparably more important.

Mean Number of Atypical Chaetae in Forked Mutants of Drosophila melanogaster

Index	Group of experiments			
	first	second	third	fourth
	♀ yeast × ♂ yeast	♀ yeast × ♂ raisin	♀ raisin × ♂ yeast	♀ raisin × ♂ raisin
No. of atypical chaetae	7.59 ± 0.19	7.58 ± 0.13	5.68 ± 0.17	4.50 ± 0.16
Difference and error of difference		0.01 ± 0.23	1.90 ± 0.31	1.18 ± 0.22

In addition to the foregoing facts, further support for this statement is given by a comparison of the scale of increasing expression of the signs of mutation in the "raisin" flies on both maternal and paternal sides (the fourth group of experiments) if one of the "raisin parents were replaced by a "yeast." If the father were replaced (third group) the result was incomparably weaker than if the mother were replaced (second group).



Relative importance of changes in diet of male and female species of the parent generation for determining the number of anomalous macrochaetae in the offspring. Along the vertical axis — mean number of atypical chaetae. Y) yeast; R) raisin diet.

In the experiments described, the nutrient medium of the females always remained unchanged throughout the experiment, whereas in the males it was changed for the two days which they spent in the same tube as the females. In the second group of experiments, for instance, the "raisin" males were transferred to a yeast medium for 2 days, during which the females laid fertilized eggs. In the third group, on the other hand, the process of copulation by the "yeast" males took place on a raisin diet. This inevitably spoiled the purity of the experiment and required the setting up of additional series of experiments in order to determine whether or not the brief period during which the males were on a different diet during the stage of copulation with the experimental females had any effect on the result of the experiment.

For this purpose, the second group of experiments was supplemented by a series in which "yeast" females were crossed, as before, with "raisin" males, but copulation with the latter took place on a raisin diet. These experiments showed no significant difference in the number of anomalous chaetae from the basic series of experiments of the second group: the mean value was 7.87 ± 0.26 macrochaetae, i.e., the difference from the value given in the table was not significant (0.29 ± 0.30). Hence, after the "yeast" females had been fed for 2 days on a raisin diet, no trace of any change in the oocytes towards normalization was found (the very small deviation in the mean number of anomalous chaetae was obviously accidental). The third group of experiments, in which "raisin" females were crossed with "yeast" males on a yeast and not a raisin diet, gave results which differed from those of the basic experiment: the resulting mean value of 6.62 ± 0.27 differed significantly from the result of the basic experiment given in the table (6.62-5.68 = 0.94 ± 0.30). The reason for this difference may be either intensification of the anomalies of the "raisin" oocytes as a result of the brief period of stay of the females on the yeast diet during copulation, or normalization of the male gametes during transfer of the males before actual copulation to the raisin diet in the second group of the basic experiment. The latter hypothesis is more in keeping with our findings in connection with the action of temperature published elsewhere [3]. The increase in the expressivity of this mutation as a result of a transient elevation of the environmental temperature took place in the females only if exposure occurred 4-10 days before the eggs were laid. If the males were exposed to the higher temperature, a slight increase in the number of anomalous chaetae was observed only if exposure took place 1-2 days before copulation. Whatever food was given to the larvae, as our previous experiments [5] showed, this had no effect on the result.

Hence, the change in the intensity of expressivity of the inherited forked developmental anomaly was mainly caused by a change in the diet of the females, i.e., it was due to some influence acting on the proembryonic period

of ontogenesis taking pace in the ovary of the maternal organism. The special importance of this period for subsequent embryogenesis has long been recognized by embryologists [1]. Recent work has shown the exceptionally great importance of injurious external influences acting in the ovarian period of development in determining the mortality of mammalian embryos during pregnancy [2, 5, 7, 8].

It is not yet understood how the nutrient medium influences the development of the gametes. It may be suggested that when flies are fed on a yeast mixture they obtain certain powerfully acting side products of metabolism, the presence of which has an adverse effect on development of oocytes. The oocytes are most susceptible to external influences at the critical period in their development, and our future researches will be directed towards the study of this problem.

We are not concerned with the question of the further transmission by inheritance of the changes in the external manifestations of inherited signs caused by a modification in the quality of the food. However, it is very unlikely that a positive solution to this problem will be found, for the agents we used have never been recorded as sources of a mutagenic action on Drosophila. The fact that changes in the phenotype of the offspring can result from the action of such weak agents as the nature of the food on their parents demonstrates what enormous resources are still available for influencing the process of development of the organism and the formation of the inherited manifestations of its individuality.

SUMMARY

An analysis was made of the early differences in the number of macrochaetae in the offspring of Drosophila melanogaster ("forked" mutant) in the culture with yeast and raisin media. The greatest expressiveness of the mutation signs was revealed in keeping both parents on yeast nutritive medium. No difference in the results was noted in crossing the "yeast" females and "raisin" males. Conversely, crossing of "raisin" females with "yeast" males resulted in a marked reduction of the number of anomalous macrochaetae. If both parents were kept on the raisin nutritive medium the offspring most closely approached the normal level. Thus, the change of the nutritive medium of the parents from yeast to raisin had a normalizing effect on the phenotype of the offspring. The shift of the manifestation of the hereditary characteristics depends mainly on the character of the mother's food. Evidently peculiarities of the female metabolism with one or another nutritive medium affect the properties of gametes developing in the ovaries.

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