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FURTHER DATA ON MATERNAL EFFECTS IN *DROSOPHILA PSEUDOÖBSCURA* HYBRIDS

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Race *B* females of *Drosophila pseudoöbscura* crossed to race *A* males produce sterile F_1 hybrid males having visibly small testes. The reciprocal cross, $A \text{♀} \times B \text{♂}$, produces in F_1 sterile males that have testes of normal size. One of us¹ has shown that this difference between the reciprocal crosses is due to a maternal effect. Before fertilization, the cytoplasm of the eggs deposited by race *B* females is so influenced by the chromosomes present in it, that an interaction between this cytoplasm and the autosomes of race *A* (introduced by the spermatozoon) results in the development of small testes in males arising from such eggs. On the other hand, the cytoplasm of an *A* race egg is so determined by the maternal chromosomes, that the introduction of *B* race autosomes results in development of large

testes. That a maternal effect, and not plasmatic inheritance, is involved in this case is proved by the fact that the further generations of the hybrids (obtained by back-crossing F_1 hybrid females to males of either parental race) are alike, irrespective of whether they carry race *A* or race *B* cytoplasm. In the present note we wish to report some further experiments bearing on the problem of maternal effects versus plasmatic inheritance.

Race *A* females carrying the second chromosome dominant Bare were crossed to race *B* males homozygous for the second chromosome recessive cinnabar. F_1 females were back-crossed to cinnabar, cinnabar females selected in the next generation and further back-crossed to cinnabar males from the original cinnabar stock for four more generations. A cinnabar stock was thus established which presumably has all chromosomes of race *B*, but whose cytoplasm is ultimately derived from the race *A* ancestor. Cinnabar flies from this stock are fertile in both sexes. To test whether the race *A* cytoplasm has any effects on the properties of these flies, such cinnabar females were crossed to wild-type race *A* males, and the size of the testes in the F_1 hybrid males was measured. As a control, cinnabar females from the original cinnabar stock (i.e., having race *B* cytoplasm) were crossed to race *A* males from the same strain as above. This test is very sensitive, since testis size in the F_1 hybrid males from the cross $B\varphi \times A\sigma$ is highly variable, and different strains of race *A*, as well as of race *B*, give quantitatively very different results.² The length of the testis in the males from the experimental and the control cultures is given below (in μ):

	$M \pm m$	σ	Limits	n
Race <i>A</i> cytoplasm	401.2 \pm 5.6	56.0	278-557	100
Race <i>B</i> cytoplasm	393.9 \pm 7.0	70.1	261-557	100

The difference between the two mean values is clearly non-significant, being smaller than its standard error. Another similar experiment was arranged by crossing and back-crossing race *A* Bare females to wild-type race *B* males (from the Seattle-4 strain) for five generations. A wild-type strain was thus obtained that should have all or most chromosomes from the race *B* Seattle-4 strain, but the cytoplasm of race *A*. Females from this strain were crossed to race *A* males. As a control, females from the original Seattle-4 strain were crossed to the same race *A* males. Testis size in the F_1 hybrids was found to be (in μ):

	$M \pm m$	σ	Limits	n
Race <i>A</i> cytoplasm	250.6 \pm 4.6	4.58	122-365	100
Race <i>B</i> cytoplasm	246.7 \pm 4.4	4.38	157-365	100

Testis size is again independent of the ultimate source of the cytoplasm. The difference between the testis size in the two pairs of experiments is due to the fact that the Seattle-4 strain is a "strong" *B* race, while cinnabar is a "weak" *B* race.²

Race *A* females carrying Bare were crossed to race *B* males homozygous for the third chromosome recessive orange. In each of the succeeding fourteen generations Bare non-orange females were selected, and backcrossed to orange males from the orange strain. Wild-type, Bare, orange and Bare orange flies appear in each generation. The strain thus obtained produces flies that have all *B* race chromosomes, except that some of them (those showing Bare) retain the section of the race *A* second chromosome carrying the locus of Bare, and some (those that are non-orange) retain the section of the race *A* third chromosome containing the locus of the wild-type allelomorph of orange. The testis size in the males in the fourteenth generation is as follows (in μ):

	$M \pm m$	σ	Limits	n
Wild-type	582.0 \pm 10.6	106.5	278-748	100
Orange	514.3 \pm 7.9	78.6	278-661	100
Bare	371.0 \pm 10.1	101.3	192-626	100
Bare orange	322.2 \pm 8.6	86.3	157-592	100

Normal males of race *B* of *Drosophila pseudoöbscura* have in similar culture conditions average testis size above 600μ , and only seldom individual males are found having testis length below 450μ . All four classes of males have, thus, testes smaller than normal. Those carrying Bare are invariably sterile, the non-Bare ones frequently so. The sterility of the Bare males shows that the section of the second chromosome of race *A* including the locus of Bare carries also a gene, or genes, whose presence in the race *B* genotype is incompatible with fertility of the males. The orange males carry, however, only race *B* chromosomes, and hence should have testes of normal size, and should be normal in fertility. This is not the case. Since, as shown above, the cytoplasm has *per se* no effect on testis size or fertility, the reduction of the testis size in the orange males must be due to a maternal effect. The presence, in a female having mostly race *B* chromosomes, of the portion of the race *A* second chromosome carrying the locus of Bare (or the portion of the race *A* third chromosome carrying the wild-type allelomorph of orange or perhaps the simultaneous presence of these two sections) modifies the cytoplasm of the eggs deposited by such a female in such a manner that the males developing from these eggs have testes smaller than normal in size even if they carry only race *B* chromosomes. The fact that no lasting or permanent modification of the properties of the cytoplasm is thus produced is, however, demonstrated by the following experiment. Orange non-Bare daughters of Bare females were crossed to orange males. All males produced in the next generation had testes of normal size. It is also established that not every section of *A* race chromosomes present in *B* race genotype produces a maternal effect. For instance, females having *B* race chromosomes except for the section of the X-chromosome carrying Pointed produce, when crossed to race *B* males, two kinds

of sons: Pointed and non-Pointed ones. The former have small testes and are sterile, the latter have normal testes and are fertile.

The offspring of the F_1 hybrid females back-crossed to males of one of the parental races consist of individuals having different combinations of the parental chromosomes. Among the males in this offspring, some have large testes and are at times fertile, others have intermediate or small testes and are sterile. It can be shown that males having all or most chromosomes of the same race have large testes, and those having a mixture of the chromosomes of both races have small testes, the smaller the more mixed are the chromosomes. However, in certain backcrosses to race B a paradoxical result was obtained: males possessing exclusively race B chromosomes had smaller testes than males having once race A third chromosome in otherwise race B genotype. This result was shown to be independent of the ultimate source of the cytoplasm, i.e., backcrosses having a race A grandmother behaved just as backcrosses having a race B grandmother did. Two explanations of this result may be suggested. First, the presence of a single race A third chromosome in a predominantly race B genotype is *per se* favorable for the testis development. Second, the race A third chromosome increases testis size in the males having mostly race B chromosomes only provided these males develop from eggs deposited by a hybrid female (which, of course, has half of chromosomes from race A , and the other half from race B). This alternative may be tested experimentally. The back-cross females having all B race chromosomes except one race A third were crossed to pure race B males. The experiment was so arranged that in the offspring the individuals having all race B chromosomes could be distinguished from those having a race A third by their phenotypes. Such an experiment showed that in the second back-cross generation largest testes are present in males having exclusively race B chromosomes. Hence, here again we are dealing with a maternal effect.³

It has been known for some time⁴ that in the offspring of the first back-cross to either race the sex ratio is abnormal in favor of females. To this should be added that many of the back-cross flies, especially among the males, are weak, visibly abnormal, and in general deficient in vigor. Females are frequently sterile. This condition contrasts with that observed in the first generation of hybrids, where the flies are more nearly as vigorous as the non-hybrid flies; the F_1 hybrid males are all sterile, but females seldom fail to produce some offspring. The following observation throws, perhaps, some light on the situation. If the chromosomes of parents are marked by mutant genes that produce no striking decrease of viability either in the pure races or in the F_1 hybrids, the back-cross hybrids, especially the males, show the Mendelian ratios for these genes grossly distorted. It appears then, that factors producing little, if any, decrease of viability in the pure races or in F_1 hybrids have a considerable deleterious

effect in the first backcross. The important point is, however, that this deleterious effect is manifested not in flies that have a particular combination of race *A* and race *B* chromosomes, but in all back-cross flies. Indeed, the classes having a given combination of race *A* and *B* chromosomes may be practically inviable with one set of marking genes, and may be represented by numerous individuals with another set of markers. The situation is best explained by assuming that the development of eggs of hybrid females is somehow disturbed by the hybrid chromosomes they carry before fertilization, and the individuals developing from these eggs are less able to withstand the effects of unfavorable factors than normal flies. In the experiments thus far done mutant genes known only in race *A* had on the whole a more unfavorable effect on the hybrids than race *B* mutants. Since, however, relatively few genes of either kind were tested, not much weight should be attached to this generalization.

As pointed out by Poulson,⁵ the time of development of the reciprocal hybrids in *Drosophila pseudoobscura* shows probably a maternal effect too. Poulson found that the larval, and especially the pupal, development of race *B* is longer than in race *A*. The time of development of the $A \text{ } \varphi \times B \text{ } \sigma$ hybrids is more nearly similar to that in race *A*, while the development of the $B \text{ } \varphi \times A \text{ } \sigma$ hybrids is more similar to that in race *B*.

Five more or less clear cases of maternal effects are, thus, established in the interracial hybrids of *Drosophila pseudoobscura*. This number seems high as compared with the situation in *Drosophila melanogaster*, where, in spite of the incomparably larger amount of genetic work done on the latter species, only a few clear cases of maternal effect are known. Whether or not this difference between *pseudoobscura* and *melanogaster* is real, and if so what is the cause of it, remains at present a matter of speculation.

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³ Experiments referred to in this paragraph will be described in more detail in a separate publication.

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