

STUDIES ON HYBRID STERILITY IV. TRANSPLANTED TESTES IN *DROSOPHILA PSEUDOOBSCURA*

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INTERSPECIFIC hybrids may be equal or superior to their parents in somatic vigor and, at the same time, show disturbances in the gonads of such a nature that no functional gametes are produced. This antithesis between the general vigor on one hand and the degeneration of the reproductive tissues on the other constitutes an interesting developmental problem. In previous papers of this series (DOBZHANSKY 1934, 1936) it has been pointed out that there are two types of hybrid sterility, the "chromosomal" type in which meiosis is abnormal because of the structural non-correspondence of the two parental sets of chromosomes, and the "genic" type in which sterility is caused by interactions of complementary genes contributed by both parents. In the chromosomal type the phenomenon of sterility is an intracellular one. In the case of genic sterility, on the other hand, it is possible, *a priori*, that intercellular influences are involved. One might suppose, for example, that there are diffusible substances, not characteristic of the parental species, produced in the hybrid, and that such substances have specific effects on the reproductive system. The work of IRWIN and COLE (1936 a, b, c) on hybrids of doves and pigeons has in fact demonstrated, by means of immunogenetic reactions, the existence in the erythrocytes of such "hybrid" substances, that is, of specific substances present in the hybrid but absent in the parents. Of course, the hypothesis of the intercellular causation of sterility does not necessarily involve the assumption of specific hybrid substances. The fate of a gonad may be determined by the general metabolism (in the broad sense of the term) of the body containing it.

Transplantation of hybrid gonads to the parental forms, and *vice versa*, provides a direct method for testing the above hypothesis. By means of appropriate transplants, it is possible to determine whether hybrid sterility, in a particular case, is determined by the constitution of the gonad itself (autonomous development), or whether the genetic constitution of the surrounding tissues is a contributing factor (dependent development). Races A and B of *Drosophila pseudoobscura* provide favorable material for such studies. The F₁ males from interracial crosses are fully viable and vigorous, but show profound and characteristic disturbances in their spermatogenesis and are completely sterile. Except for the testes, the reproductive system appears to be quite normal.

MATERIAL AND METHODS

The technique of transplantation developed by EPHRUSSI and BEADLE (1935, 1936) for *Drosophila* has been used successfully by these workers for transplanting larval testes of *D. melanogaster* (unpublished). In case of *D. pseudoobscura* special thin-walled large-bore micro-pipettes were employed, but otherwise the technique was essentially the same as described for *D. melanogaster*. Larval testes must be handled with a good deal of care to avoid rupturing the delicate external membrane. Unless otherwise stated, the transplantations described below were made with larvae approaching the prepupal stage.

Four strains of *D. pseudoobscura* were used: wild type strains Zuni-5 (race A, strong), and Seattle-6 (race B, moderately weak), and mutant strains eosin magenta short (race A, rather strong), and scutellar dela (race B, intermediate). The two latter strains carry mutant genes affecting the eye color as well as that of the testicular envelope. The experiments were so arranged that it was possible to distinguish the implant from the testes of the host by color. Testis color in these experiments proved to develop autonomously, although non-autonomous development of this character has been observed in *D. simulans* (DOBZHANSKY 1931).

Eggs were collected over 24 hour periods at room temperature (about 22°C), then allowed to develop and hatch at 25°. Approximately 48 hours after collection, larvae were transferred to culture dishes and, from that time until transplantations were made, were grown at 19°. Larvae on which operations had been made were allowed to develop to maturity at 25°. In many cases, it was necessary to provide food after the operations; this was done by adding to the vial a small piece of standard food seeded with yeast.

The implanted testes, and in some cases those of the host, were fixed and stained in aceto-carmin, and smear preparations made. Spermatogenesis in normal and hybrid males has been described previously (DOBZHANSKY 1934). The difference in spermatogenesis between hybrid and normal males is characteristic enough, so that relatively slight modifications of either are readily detectable. In the hybrids the chromosomal pairing at meiosis is more or less suppressed, no second spermatocytes are formed, and the spermatids undergo a characteristically abnormal development. All of these abnormalities were looked for in preparations of the implanted testes and in the testes of the host, especial attention being paid to chromosome pairing at diakinesis and metaphase of the first meiotic division, since the process of pairing is known to be sensitive to external agents (BAKER 1936, BAUER, unpublished).

DEVELOPMENT OF IMPLANTED TESTES

Among 146 individuals in which testes were implanted in the larval stage, 22.8 per cent contained no implant when dissected, presumably because of loss or injury to the testis during the process of injection. Among those flies in which the implant had developed 61.1 per cent had the implant lying in the body cavity not connected with the reproductive organs of the host except by tracheal branches, 27.3 per cent had the implanted testis attached to one of the two vasa efferentia and one of the host's testes free in the body cavity, and in 11.5 per cent both the implant and one of the host's testes were attached to the same vas efferens (fig. 1).

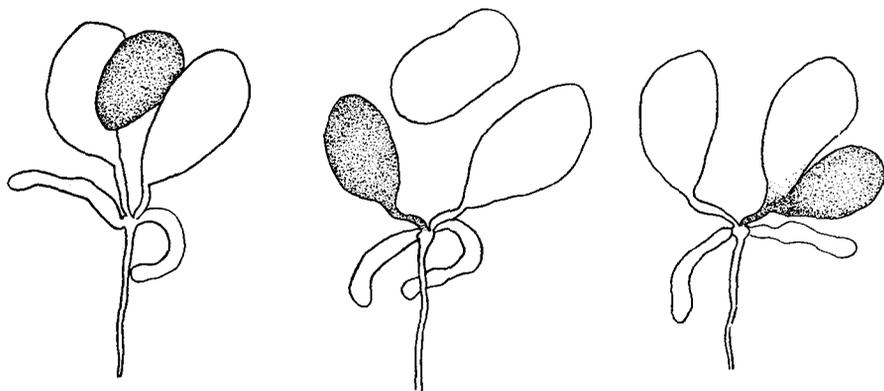


FIGURE 1.—The internal reproductive organs of eosin magenta males with A ♀ × B ♂ hybrid testes implanted in them. The implanted testes are in this case red (stippled in the figure), and those of the host pale yellow (white in the figure). Left—both host's testes attached to the vasa efferentia, the implanted testis free. Middle—the implant attached, one of the host's testes free. Right—implanted and host testes attached to the same vas efferens.

The connection established between the implanted testis and the vas efferens of the host may become functional, as shown by the fact that the sperm from the implant may be used in fertilization (see below). When two testes are attached to the same vas efferens it is not known whether the connection is functional for both of them. The data indicate that race A testes implanted in race B hosts become attached to the ducts of the host more frequently than race B testes in race A hosts; however the numbers of individuals are so small that this conclusion cannot be established with certainty. In the hybrids in which the testes of the host are small and the implanted testis of one of the pure races is much larger, no tendency is observed for the implant to be attached in preference to the host's testes.

In a control experiment testes of eosin magenta short (race A) were implanted in Zuni-5 (race A) larvae. Spermatogenesis in the implanted testes was found to be normal, showing that the process of transplantation as

such does not affect the normal development of the testis. During the entire study preparations of testes found lying free in the body cavity were kept separate from those of testes attached to the ducts; no difference was found in spermatogenesis in these two classes. Furthermore, in some cases testes were implanted into female hosts. A testis which develops in a female body is somewhat smaller in size than one which develops in a male. A similar observation was made in the case of certain types of gynandromorphs in *D. simulans* (DOBZHANSKY 1931). In case of attachment to a female duct no study of spermatogenesis was made, but no abnormalities in spermatogenesis were detected where the testis was not attached to an oviduct.

TRANSPLANTS BETWEEN RACES

Nine Zuni (race A) males with dela (B) testis implants, and seven eosin magenta (A) males with Seattle (B) implants were studied cytologically. Spermatogenesis was found to be quite normal in all stages. In 215 first spermatocytes, the normal number of bivalents, namely four, was observed. *D. pseudoobscura* has five pairs of chromosomes, but the fifth pair is so small that the bivalent formed by it is not usually seen at the meiotic stages. In one cell the X and Y chromosomes were unpaired, but such failure of pairing is occasionally observed in normal testes (DARLINGTON 1934). In the testes of the host spermatogenesis was observed to be normal. In 85 first spermatocytes, four bivalents were observed in each, and in one cell there were three bivalents and two univalents (X and Y chromosomes). One cyst of tetraploid spermatocytes was found. Six dela (B) males with Zuni (A) implanted testes also showed normal spermatogenesis in both the host and the implant. Four bivalents were seen in 99 first spermatocytes in the implanted testes and in 29 spermatocytes in the host.

In order to demonstrate that spermatogenesis is not only visibly normal but also leads to the production of functional sperm, eosin magenta males with Seattle implants were crossed to eosin magenta females. Nine out of ten such crosses produced offspring, which in six cases consisted of eosin magenta individuals only, and in three cases of eosin magenta as well as wild type individuals. Clearly, in the six former only the testes of the host, and in the three latter both the host's and the implanted testes functioned. Dela males with Zuni testes implanted were crossed to dela females; one out of four cultures produced only dela flies, and the remaining three both dela and wild type flies. The cases where no offspring were produced from the implanted testes are presumably those in which the implanted testis did not become attached to the vasa efferentia. The conclusion is justified that a race A testis developing in a race B body neither loses its ability to

function nor produces any disturbance in the testes of the host. The same is true for race B testes implanted in race A.

IMPLANTS FROM PURE RACES TO HYBRIDS

Four experiments of this type were made: (1) eosin magenta A testes were implanted into Zuni A ♀ × Seattle B ♂ hybrids, (2) dela B into Zuni A ♀ × Seattle B ♂, (3) eosin magenta A into Seattle B ♀ × Zuni A ♂, and (4) dela B into Seattle B ♀ × Zuni A ♂. In race B ♀ × race A ♂ hybrid males, the testes are visibly smaller than in either pure race or in A ♀ × B ♂ hybrids. Therefore, in the third and fourth experiments the implanted testes proved to be much larger than those of the hosts, while in the first and second experiments the implants were of the same or of slightly smaller size than those of the hosts. The implanted testes were studied cytologically and all stages of spermatogenesis proved to be normal. In the first experiment ten testes were examined and 54 first spermatocytes with four bivalents in each were seen; in the second experiment eight testes were examined and 68 spermatocytes with four and 1 with three bivalents (X and Y unpaired) found; in the third experiment five testes were examined and 76 cells with four bivalents found; in the fourth experiment eight testes were examined and 49 cells with four and 2 cells with three bivalents seen (X and Y chromosomes unpaired in one cell and an autosome unpaired in another).

About 12 males from each experiment were crossed to females homozygous for the recessive genes carried by the implanted testes. Since the hybrid males are normally sterile, the production of offspring from these matings would mean either that the implanted testes are able to function or that the presence of the implant induces fertility in the testes of the host. The presence of mutant genes as markers enables one to discriminate between these possibilities. Two males from each of the first, third and fourth experiments proved to be fertile, and the offspring showed that the sperm must have come from the implanted testes. We can conclude: (1) that the functioning of the testes of the pure races is not interfered with when they develop in the body of a hybrid, (2) that the testes of a hybrid host do not become fertile in the presence of a normally developed implant, and (3) that the ducts of the reproductive system in the hybrid males are normal and potentially functional.

IMPLANTS FROM HYBRIDS TO PURE RACES

Four experiments were made in which testes from hybrid males were implanted in males of pure races: testes of Seattle B ♀ × Zuni A ♂ hybrids in (1) eosin magenta A males and (2) dela B males, and testes of Zuni A ♀ × Seattle B ♂ hybrids in (3) eosin magenta and (4) dela males. In all

cases autonomous development of the implant was observed. The B ♀ × A ♂ hybrid testes were small, and those of the A ♀ × B ♂ hybrids were about equal in size to testes of the pure race hosts. In the first, second and fourth experiments seven implants were studied cytologically; in the third experiment three testes were studied. Spermatogenesis was found to be similar to that normally observed in hybrid testes; disturbance or complete lack of meiotic pairing, absence of second spermatocytes, degeneration of the spermatids, and other characteristic abnormalities. Four testes of the hosts were also examined, and found to contain normal stages of spermatogenesis. Five males from the first experiment were tested for fertility, and all proved to be fertile. It follows that the presence of a hybrid testis in the body of a normal male induces no change either in the implant or in the host.

TRANSPLANTATION AT EARLIER STAGES

In the experiments described above, both the donors and the hosts were nearly mature larvae (since the results were uniform, data regarding the precise ages of larvae in each individual experiment have been omitted). We have seen that under these conditions the development of the implanted as well as of the host testes is autonomous. It follows that in the mature larvae the fate of the testis is already determined, in the sense that its transplantation into the body of a foreign race does not produce any effect on spermatogenesis. The possibility remains however that if the transplantations were made at an earlier stage the results would be different. As an analogy, the findings of BAKER (1935) may be mentioned here. BAKER has shown that there is a sensitive period for the influence of high temperature on the testes of *D. pseudoobscura*, and that this sensitive period lies in the prepupal stage, that is, precisely the stage at which the majority of our transplantation experiments were made.

In the following two experiments the donor and the host larvae were much younger than in those experiments reported above, namely three days before pupation, which corresponds to the end of the second or the beginning of the third larval instars. After the operations were made, the larvae were given food sufficient to cover the period to pupation. The experiments were: (1) implants of Zuni A and (2) of Seattle B testes in *delaware* B ♀ × Zuni A ♂ hybrid larvae. Cytological examinations were made on five testes from the first experiment and on seven testes from the second. All stages of spermatogenesis were found to be normal: 72 spermatocytes in the first and 64 in the second experiment were found to contain four bivalents each; one testis from the first experiment contained a cyst of tetraploid cells, and in another testis a spermatocyte with only three bivalents (X and Y unpaired) was found.

About a dozen males from each of the two experiments were tested for fertility by crossing them to eosin magenta and to dela females respectively. Three males from the first, and none from the second experiment proved fertile. This low frequency of fertile males is due to the fact (ascertained by dissection) that in the large majority of males the implanted testis was not attached to the vasa efferentia.

DISCUSSION

Only few transplantation studies have been made which bear on the question of hybrid sterility in other insects. BYTINSKI-SALZ (1933) transplanted the ovaries of the pupae of the hybrid *Celerio euphorbiae* ♀ × *C. gallii* ♂ into pupae of the parental species. Normally the ovaries of the donor do not develop to maturity, since the hybrid does not survive the pupal stage; but the transplants were observed to grow and to form nearly mature eggs. It remains unclear whether in these experiments a transformation of a sterile gonad into a fertile one is involved, or (which seems vastly more probable) merely a survival of an organ which would normally die together with the organism containing it. EPHRUSSI and BEADLE (1935) transplanted the ovaries of *Drosophila simulans* to *D. melanogaster*, and found that the implant produces functional ova (the hybrid between these two species is completely sterile). Thus, the development of the ovary of one species in the body of another does not lead to a condition resembling hybridity. EPHRUSSI and MONOD (unpublished) transplanted the ovaries of *D. melanogaster* into *melanogaster* × *simulans* hybrid larvae. The resulting adult flies have three ovaries; two ovaries of the host are rudimentary (as they normally are in the hybrid), while in the implanted ovary apparently normal ova are present. The conclusion is that the ovary of *melanogaster* is not affected by development in the hybrid organism.

The data presented in the present paper are consistent throughout. The testes of one pure race develop normally in the body of the other race and in the hybrids, and the hybrid testes develop autonomously in the body of either race. Cytological investigation shows that there is no detectable influence of the host on the implant. Pure race testes produce functional sperm in the hybrid body, and the hybrid testes never develop fertility when implanted into the pure races. The development of the testes is autonomous, "herkunftsgemäss" and not "ortsgemäss." It follows that the condition of the gonad is determined by the genetic structure of the cells within the gonad and not by interactions between the gonad and influences emanating from other parts of the body. The only escape from this conclusion is to suppose that in our experiments the transplantations were made after the period during which the gonad may be sensitive to the influences of other parts of the body has passed. Such a supposition

has little chance to be correct, not only because some of the experiments were made in rather young larvae, but also because in *D. pseudoobscura* the proliferation of the primary spermatogonia continues during the whole of the pupal and large part of the adult life. At the stage at which the transplants were made the gonads contain mainly spermatogonia and a few spermatocytes. In other species of *Drosophila*, notably in *melanogaster* and *simulans*, the situation is different in this respect.

The phenomenon of sterility in the interracial hybrids in *D. pseudoobscura* must be intragonadal in nature. It would seem that only those external influences can modify the behavior of the sex cells which can act directly on the gonads. No "somatic induction" is likely to occur. This consideration is of importance for planning experiments which have to do with the physiology of sterility. It may be noted here that in cases where the sterility is of the chromosomal type (DOBZHANSKY 1934, 1936) the phenomenon is almost of necessity an intracellular one; but where genic sterility is concerned both intra- and intercellular causations are *a priori* equally possible. Hence, the results of this investigation of *D. pseudoobscura* cannot be generalized further. It should also be noted that transplantation of gonads between species may be expected on *a priori* grounds to produce in some cases a suppression of the implants. Such a suppression may not mean however that a condition resembling hybrid sterility is induced, unless it is demonstrated that cytological abnormalities resembling those found in sterile hybrids are apparent.

SUMMARY

Larval testes of race A were implanted into race B larvae, and *vice versa*. Testes of A×B hybrids were implanted into the pure races, and pure race testes were implanted into hybrids. Study of spermatogenesis has shown that the development of the implanted testes as well as of those of the host is autonomous in all cases. It follows that in *Drosophila pseudoobscura* the structure of the gonad is determined by its own genetic constitution and not by the genetic constitution of the surrounding soma.

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