SPECIFIC REACTIONS OF THE OVARY TO INTERSPECIFIC TRANSPLANTATION AMONG MEMBERS OF THE MELANOGASTER GROUP OF DROSOPHILA

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INTRODUCTION

The transplantation of ovaries in Drosophila was realized for the first time by EphruSSI and Beadle (1935b), using the technique which they had devised and applied with such success to the transplantation of the eye discs and other anlagen. Their results showed that the ovaries of D. melanogaster can be successfully implanted into male and female larvae of the same species, can become attached to the female genital ducts of the host, and can function almost as efficiently as the host ovaries themselves. In some cases as many as three ovaries were transplanted to a single female and developed successfully, together with the host ovaries. Furthermore, EphruSSI and Beadle showed that interspecific transplants were possible, at least in the case of simulans ovaries in melanogaster. These implants proved to be quite as successful as the homospecific ones, for simulans ovaries became attached to the genital ducts and functioned normally in a melanogaster host.

It was obvious that by using this technique many crosses which are normally impossible could be attempted, since in most cross combinations no mating occurs for lack of sexual attraction or for other reasons. Studying the possibilities of such a technique was the first aim of the experiments presented here. The results of these experiments, however, brought evidence of some interspecific reactions of a particular kind, which are limited to the ovary and depend, not so much on the general constitution of the species used, as on the action of certain genes. These phenomena seem to set an entirely new problem, the nature of which will be discussed in this paper.

The experiments were originally begun by one of us (J. Monod) with Dr. Boris EphruSSI, but were carried to completion by both authors. Acknowledgment is made to Dr. EphruSSI for his part in the work and especially for his stimulating interest, and to Mr. C. W. Clancy and Dr. G. W. Beadle for consenting to the presentation of certain of their unpublished data.

TECHNIQUE AND MATERIALS

The transplantation technique of EphruSSI and Beadle (1936) has been used by us without change, together with their methods of raising larvae.

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Unless specifically indicated all of our transplants have been made between larvae ready to pupate.

The larval ovaries which can be distinguished from the testes by their smaller size (Kerksis 1931) were dissected out in Ringer's solution and all but a small portion of the fat body was removed before injection. At this time the ovaries have attained only a fraction of the size of the imaginal ovaries and are actively growing. Differentiation of the ovary occurs in the pupal and the early imaginal stages. In melanogaster and simulans the ovaries are mature and contain eggs within two days after the eclosion of the imago, and in annanassae within five days. The life cycle is somewhat longer in annanassae than in melanogaster and simulans (Moriwaki 1935). The mature imaginal ovaries of the three species are of approximately the same size and occupy about the same proportion of the body cavity. At that time the ovaries are many-chambered and are filled with oocytes and eggs. The time required for the ovaries to mature was taken into account when the hosts were dissected. In the case of the melanogaster implants the hosts were well fed and aged for at least two days; in the case of annanassae implants, for at least five days. Hosts were then dissected and comparison of implanted and host ovaries made. Any further aging was found to be unnecessary.

Most of our experiments deal with reciprocal transplants between D. melanogaster and D. annanassae. D. annanassae belongs to the melanogaster group and, according to Prof. A. H. Sturtevant, seems to be very closely related to D. melanogaster. D. caribbea is a synonym. The strain used was of Japanese origin. The standard wild stock of D. melanogaster used was that known as Oregon R.

**Experimental Results**

Table I summarizes our results on ovary transplants together with some results obtained by Ephrussi and Beadle (1935b) and by Clancy and Beadle (unpublished). In the first column is listed the species or strain of the implanted ovary and in the second column that of the host. The conditions of the ovaries at the time of dissection are given in the last three columns: rudimentary ovaries having but a single chamber, the most extreme; large ovaries with several chambers containing many oocytes, but no eggs; and ovaries with eggs as well as large numbers of oocytes. The differences in sizes between the first two classes are enormous; between the last two, comparatively slight. All ovaries observed fell within one of the three categories. There were no doubtful cases.

When annanassae ovaries are transplanted to melanogaster hosts the growth of the implant seems to be almost totally inhibited. After dissection the ovary appears as a very small mass, smaller in most cases than the
rudimentary ovaries of *melanogaster × simulans* hybrids (Kerkis 1933). It consists of one chamber only, very rarely of two. Because of its small size there is frequently difficulty in locating the implant. The same result is obtained when *annanassae* ovaries are transplanted to *simulans* hosts, both with respect to the size of the ovary and its stage of development.

<table>
<thead>
<tr>
<th>IMPLANT</th>
<th>HOST</th>
<th>RUDIMENTARY OVARIIES</th>
<th>OVARIIES WITH SEVERAL CHAMBERS</th>
<th>OVARIIES WITH EGGS</th>
</tr>
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<tr>
<td><em>annanassae</em></td>
<td><em>melanogaster</em></td>
<td>11</td>
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<td>0</td>
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<td><em>simulans</em></td>
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<td><em>mel.×sim.</em></td>
<td>0</td>
<td>4</td>
<td>16</td>
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<td>♀ st/♀ st (<em>melanogaster</em>)</td>
<td>3</td>
<td>3</td>
<td>7</td>
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<td>♀ st/Cy (<em>melanogaster</em>)</td>
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<td>0</td>
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<tr>
<td><em>melanogaster</em></td>
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<td>3</td>
<td>16</td>
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<td><em>melanogaster</em></td>
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<td>4</td>
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<tr>
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<td>1</td>
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<tr>
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<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>♀ <em>melanogaster</em></td>
<td>♀ st/♀ st</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>†♀ st/♀ st</td>
<td><em>melanogaster</em></td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Data of Ephrussi and Beadle (1935).
† Data of Clancy and Beadle (unpublished).

On the other hand *melanogaster* ovaries transplanted to *annanassae* develop normally, with few exceptions. Of the 27 cases only three failed to develop beyond the rudimentary stage. As far as could be determined, these were comparable to the *annanassae* ovaries in *melanogaster* and in *simulans*. All of the remaining ovaries were large with many oocytes, and eight (one-third) of them had nearly mature eggs. In the case of *simulans* ovaries in *annanassae* no rudimentary ovaries were obtained. The number of ovaries with nearly mature eggs is much below that of the large ovaries with many oocytes, indicating a slight effect. The homospecific transplants of *annanassae* ovaries in *annanassae, simulans in simulans,* and *melanogaster in melanogaster* always develop into mature ovaries with eggs. Thus effects resulting from the operation itself are eliminated. For this reason the two classes of many-chambered ovaries are significant and indicate a small effect of *annanassae* as host. However, the notable effect, which is the inhibition or complete failure to grow, is a "one way" effect.

The development of the transplanted ovary is the same regardless of the sex of the host. As many cases of “inhibition” were obtained in male hosts.
as in female. Attachment of the ovary to the female genital ducts is without effect one way or the other. The transplant may or may not become attached. The frequencies of attachment which we observed agree with those obtained by Ephrussi and Beadle (1935b). Thus competition between host and implanted ovaries is eliminated. No cases of fusion of the transplant with a host ovary were observed.

No influences of the transplant on host ovaries were observed. Preliminary experiments showed no such effect when two ovaries were injected into the same host.

The question arises as to whether or not the effect is to be found in the testis as well as the ovary. A great many testis transplants showed that while there is an effect on the size of the testis in both directions, the effect is not comparable at all with the effect on the annanassae ovary. In all cases of transplanted testes mature spermatozoa were present. Annanassae testes implanted into melanogaster are always undersized when compared with normal annanassae testes and do not generally assume the shape of normal testes. The result of control implantation of annanassae testes into annanassae is about the same although the size attained by the implant is somewhat greater. Thus the observed inhibition is specific with respect to the ovary and does not belong to the general category of plasma and tissue incompatibilities of which so many instances already exist (Loeb 1930).

In an attempt to determine whether there is an effect of the age of the host, ovaries from annanassae larvae ready to pupate were injected into melanogaster hosts twenty-four hours younger. Here, as in the first experiment, the annanassae implants were always rudimentary.

It seems possible that the phenomenon may be connected with some specific mechanism of ovarian development. In that case, the annanassae ovary might be expected to grow to some extent in hosts in which the development of the ovaries is abnormal. The melanogaster × simulans hybrids constitute a good case of this kind, the ovaries here being reduced to rudimentary proportions (Sturtevant 1920, Kerakis 1933).

The result of the transplantation of annanassae ovaries to hybrid hosts is striking. Of the 20 successful transplants 16 were completely developed ovaries with eggs. The remaining four were completely developed but without eggs. There was no "inhibition."

The behavior of annanassae ovaries in melanogaster × simulans hybrid hosts might suggest that the "inhibition" has its origin in some species difference which would be less marked in the hybrid than in the pure species. Such an assumption, however, is in contradiction to the results of the transplantation of annanassae ovaries into female-sterile melanogaster (♀st in chromosome II). The homozygous ♀st females are completely
sterile and their ovaries are considerably reduced, although not so much as in the melanogaster X simulans hybrids. For our purposes females carrying ♀ st in one chromosome and Curly in the other were mated to homozygous ♀ st males. The heterozygous females are fertile, and the males, both hetero- and homozygous for ♀ st, seem to be perfectly fertile. The results here, though somewhat variable, show that the transplanted annanassae ovaries may grow to considerable proportions in homozygous ♀ st hosts, although totally inhibited in the heterozygote. Here again the results are the same whether the host is male or female.

The effect of host on transplant is not necessarily related to the sterility as such, of the host, but to the conditions of growth of the ovary. In melanogaster homozygous for the mutant character singed (sn) the ovaries are fully developed though their products are sterile. Annanassae ovaries, transplanted to male and female singed, showed that singed behaves in that respect like the normal wild type.

The data of Clancy and Beadle (unpublished) included in table I show that wild type melanogaster ovaries transplanted to female-sterile hosts develop normally, while female-sterile ovaries in wild type melanogaster remain rudimentary. Likewise the hybrid ovary of melanogaster X simulans fails to develop any further when placed in a melanogaster host. Thus it appears certain that the development of the ovary is determined at earlier stages than have been dealt with in these investigations. Dobzhansky and Beadle (1936) found a similar situation in the case of transplants of the testes of the two races of D. pseudoobscura. Testis development was autonomous in all cases.

In the case of the annanassae ovary in melanogaster or simulans, however, the presence or the absence of some substance appears to be essential in the pupal and early imaginal stages.

DISCUSSION

The experiments which have been described eliminate the effects of competition between ovaries as well as direct effects of the host ovaries upon the transplants. They show that the phenomenon in question is limited to the ovary. Likewise it is evident that behavior of the annanassae ovary in both melanogaster and simulans hosts is of quite a different nature than that of the melanogaster X simulans hybrid ovaries or the female-sterile ovaries.

It is difficult to determine from the data whether the inhibition is of a positive or negative nature, that is, the result of the presence or absence of a particular substance. It is possible, however, that a substance produced in the annanassae larva in addition to causing the annanassae ovary to develop through the later stages is quite as effective in cases of melano-
gaster and simulans ovaries transplanted to annanassae hosts. On the other hand the substance which is present in melanogaster and annanassae larvae may be sufficiently different so that the annanassae ovary cannot complete development in either of those hosts.

To account for the development of annanassae ovaries in the melanogaster × simulans hybrid and female-sterile hosts it is necessary to assume that the ovary stimulating substance produced there is the same as, or similar to, that of the annanassae larva. Thus we have a consistent, if hypothetical, explanation.

Such an hypothesis becomes more plausible in the light of recent work on the physiology of insects. That a certain substance is essential for the complete development of the insect ovary has been strikingly demonstrated by Wigglesworth (1936) who found that in Rhodnius and certain other Hemiptera the corpus allatum secretes into the haemolymph a substance, "hormone," controlling the development of the eggs in the ovary. In the male this substance affects the accessory glands, but is not essential for the production of spermatozoa. The substance is the same in both sexes. Thus the fact that the results of our experiments were identical, regardless of the sex of the host, is clear, as is the fact that in insects in general gonads may be successfully transplanted from one sex to the other.

In line with this are the experiments of Bytinski-Salz (1933) who successfully transplanted larval ovaries of a Lepidopteran hybrid to hybrid male larvae. In the cross the females died as larvae, and only the males reached the imaginal stage. Nevertheless the transplanted ovaries developed in the male hosts.

Whatever the mechanism may be, the inhibition itself does not appear to depend on species constitution in general, but rather on genic differences. In the case of female-sterile the difference, as far as we know, is that of a single gene.

SUMMARY

1. Ovaries of D. annanassae transplanted to melanogaster and to simulans hosts fail to develop normally and remain rudimentary.
2. Ovaries of melanogaster and simulans transplanted to annanassae hosts develop almost completely normally, in many cases producing eggs and in all cases many oocytes.
3. Ovaries of annanassae transplanted to melanogaster × simulans hybrid hosts and to homozygous female-sterile melanogaster hosts develop almost completely normally.
4. The reaction of the annanassae ovary is shown not to be due to general interspecific tissue incompatibilities, but to be dependent on genic constitution.
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