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Citation	北海道大學理學部紀要, 10(2), 127-134
Issue Date	1951-03
Doc URL	http://hdl.handle.net/2115/27086
Type	bulletin (article)
File Information	10(2)_P127-134.pdf



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**Testicular Transplantation in Grasshoppers, with Special
Remarks on Cytological Phenomena in the Grafted
Testes¹⁾ (Studies on Abnormal Nuclear Divisions, 4)**

By

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(With 1 Plate)

With prime interests directing towards the effects on secondary sexual characters and sex transformation, experiments of the gonadal transplantation as well as gonadectomy have been exclusively done in higher animals such as mammals, birds, amphibians and fishes, and a number of important papers have been published in this field, with excellent results on the respective articles. Very little progress has been made, however, concerning the related subjects in invertebrates, specially in insects, and very few contributions have made in connection with mainly the question whether the similar phenomena as found in higher animals do occur in such lower animals too (Meisenheimer 1909, Regen 1909, Kopeč 1911, Klatt 1919, Umeya 1928, Wigglesworth 1936, Pfeiffer 1939, Pflugfelder 1939, Vogt 1940, Fukuda 1941). In the present paper the author wishes to present the results from histological and cytological observations on testicular grafts of some grasshoppers implanted in the body of female. Under the poor status of study in this field, therefore, to publish this work might not be entirely uncalled for, though the investigation may not be fruitfully worked out.

The author takes much pleasure in expressing his cordial thanks to Professor Sajiro Makino under whose suggestion and guidance the work has been carried out. Thanks are also due to Professor T. Uchida of this Institute and Professor K. Takewaki of the Tokyo University for their helpful advices.

Material and Methods

The present experiments were carried out with the following four species of

1) Contribution No. 244 from the Zoölogical Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

Aided by the Scientific Research Fund from the Ministry of Education.

Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 10, 1951.

grasshoppers belonging to the Acrididae: *Podisma sapporensis*, *Eirenephilus longipennis*, *Miramella mikado* and *Oxya jezoensis*.

The testes of these grasshoppers are composed of a certain number of elongated testis-follicles, each being of banana-shape and connected with a common duct at their distal ends. The testes were removed from the abdomen by vivisection from nymphs as well as from adults, and immediately grafted in the abdomen of the female or male bodies, in which the gonads were left untouched. In grafting, the whole of the testis was put with the aid of a fine forceps into the abdominal cavity of the host animal, without utilizing narcotics, through a thin slit made at the dorsal left side. The wounds were not closed by artificial ways, but the clotting of the blood over the wounds eventually prevented further escape of blood. At moult, however, a considerable number of operated animals died as the result of difficulty in shedding the old cuticula at the wound.

The operated specimens were reared for necessary length of time to research the external bodily changes and behavior, feeding with fresh grass leaves. After a period of 10, 20 and 30 days they were killed, and the grafted gonads were taken out and fixed with Allen-Bouin's solution. The gonads were prepared according to the usual paraffin method, and the sections were stained with Heidenhain's iron-haematoxylin for cytological study. As control, the gonads obtained from animals reared as long as the operated animals under the same condition, were examined after the same method under microscope. The general scheme of the present experiments is given in Table 1.

The operation was performed in early summer of 1949 and 1950.

Table 1. Scheme of experiments.

No. of Exp.	Hosts			Testes to be grafted		Number of indiv. operated	Days reared	Number of indiv. survived
	Species	Sex	Age	Species	Age			
I	<i>P.s.</i>	♀	Ad.	<i>P.s.</i>	L.(3-4M.)	35	10	1
II	"	"	L.(1-2M.)	"	L.(1-2M.)	29	30	1
III	<i>M.m.</i>	"	L.(3-4M.)	<i>M.m.</i>	L.(3-4M.)	25	20	2
IV	<i>P.s.</i>	"	Ad.	<i>E.j.</i>	"	20	10	1
V	"	"	"	<i>M.m.</i>	"	31	10	2
VI	<i>O.j.</i>	"	"	<i>O.j.</i>	"	21	10	3
VII	"	"	"	"	"	28	20	2
VIII	"	"	"	"	"	25	30	2
IX	"	♂	"	"	"	30	10	3
X	"	"	"	"	"	28	20	2

Remarks: *P.s.*=*Podisma sapporensis*. *M.m.*=*Miramella mikado*. *E.j.*=*Eirenephilus longipennis*. *O.j.*=*Oxya jezoensis*. Ad.=Adult. L.=Larva. M.=Moulting.

Observations

1. Morphological structure of implanted testes

i) *Intraspecific transplantation*: Reciprocal homoplastic transplantations of testes were undertaken between different individuals of *Podisma sapporensis*, *Miramella mikado* and *Oxya jezoensis* respectively (Exp. No. I-III, VI-X of Table 1). The scheme of the transplantation is as follow:

Table 2

Testes of	implanted in
i) <i>P. sapporensis</i>	Female bodies
ii) <i>M. mikado</i>	„
iii) <i>O. jezoensis</i>	„
iv) „	Male bodies

The operated animals were reared for a period of 10, 20 and 30 days and then killed. So far as the scope of the present observations is concerned, the testicular transplantation in the body of a female brings about no modification of the sexual characters of the latter. At autopsy, it was shown that in every case the grafted testes exhibited nothing abnormal in their external feature being nearly similar to those of the control animals, except that the follicles were somewhat shorter in length.

Histological observations made with sections revealed that there had occurred a remarkable morphological change of the epithelial tissue covering the testis-follicles in the testes located in the female bodies. The follicle epithelium, which consists of a thin membranous layer in the normal testis, showed pronounced or sometimes an unusual outgrowth in grafted testes, as seen in Figs. 2 and 3. The abnormal overgrowth of the follicle epithelium seems to have increased in degree with the time elapsed since the implantation. The testes of a longer implantation showed in general abnormal follicles with adhering by unusually thickened epithelium and filled with germ cells being mostly in a stage of degeneration. Such an abnormal growth of the follicle epithelium was hardly found in the testes grafted in the male individuals (Fig. 1).

ii) *Interspecific transplantation*: The testes of *Eirenephilus longipennis* and also those of *Miramella mikado* were transplanted in female bodies of *Podisma sapporensis*. The grafted testes were left for 10 days in the bodies of the hosts before autopsy (Exp. No. IV and V of Table 1). On examination it was surprising to find that the testicular grafts underwent, uniformly in both cases, a striking rudimentary change in external structure. The testis-follicles of *E. longipennis* implanted in female bodies of *P. sapporensis* were found ill developed, being small in size. Those of *M. mikado* located in the female of *P. sapporensis* became highly disorganized, showing a remarkable decrease in size in one of two cases here observed, and in the

other one they were found as transparent bodies of very slender form.

As occurred in the previous case, the testicular transplantation gave rise to no modification of the sexual character of the host in the present experiment too.

Histological examination revealed that the testis-follicles had become highly disorganized showing a remarkable shrinking. The germ cells contained were mostly in process of degeneration and lacked a spermatogenetic activity at all. In the extreme case the follicles contained no germ cell with normal feature.

2. Cytological features in grafted testes

Cytological observation detected the fact that in every case here concerned there were observed a number of atypical spermatocytes and their abnormal nuclear divisions in the follicles of implanted testes, in both the cases of the intraspecific and of the interspecific transplantation.

The fusion or clumping together of chromosomes is one of the most frequent abnormalities found in the grafted testes (Fig. 6). The fusion is usual in metaphase chromosomes, but sometimes occurs in chromosomes of the growth period. The fusion of chromosomes is probably caused by stickiness of the matrical substance. The formation of chromosome-bridges which is frequently observable and be attributable to sticky chromosomes (Fig. 5). The atypical arrangement of chromosomes at metaphases is also common. The chromosomes lie scattering on the equatorial plane instead of normal radial arrangement, or clumping together occupying an eccentric area of the plate. The irregular separation of chromosomes at anaphase or migration in different numbers to poles is also striking (Fig. 4), showing some fragmental elements on the way of migrating to poles. It is evident that most of these abnormalities may result in the production of nuclei containing varying chromosome contents.

The pycnotic aggregation of chromosomes often observed may be an extreme case of chromosome coalescence (Figs. 8 and 9). The abnormal swelling of chromosomes is also one of the noticeable changes observed. The advance of swelling may result in the deformation of chromosomes into usually rounded bodies.

Bi-nucleate and tri-nucleate cells are a rather abundant phenomenon usually observed in degenerating follicles (Figs. 7 and 8). The nuclei contained are of nearly similar size in most cases, while in a few cases they are dissimilar in size. It seems likely that the origin of these multi-nucleate cells is to be regarded as one of two events; namely fusion of two or three cells or nuclear division without cell-division. Very often each of the nuclei contained is found as a compact pycnotic ball, while sometimes one of them assumes a regular appearance.

The frequency of occurrence of mitotic abnormalities as above described was observed in the testes implanted for 10, 20 and 30 days respectively. The results of observation indicate that the mitotic abnormalities show a general tendency to decrease with the time of transplantation. In other words, the abnormal figures found in testes of a 30-day-implantation are on the whole less in number than those

found in testes of a 10-day-implantation. Figs. 10 and 11 give chromosome figures apparently regular in shape and behavior during the meiotic divisions, which had taken place in the testes of *Oxya jezoensis* left for 30 days in female bodies. The probable interpretation for this phenomenon seems to lie in the fact that by a longer time of transplantation a sufficient nutrition necessary for development of cells would be maintained by means of some successful connection of tissues having taken place between the graft and the host.

Remarks

There have appeared some papers involving experiments of gonadal transplantation in insects, published by Meisenheimer (1909), Kopeč (1911) Regen (1909), Prell (1915), Klatt (1919), Umeya (1928), Wigglesworth (1936), Pfeiffer (1939) Pflugfelder (1939), Vogt (1940) and Fukuda (1941). They have been mostly carried out with lepidopterous insects as material, with particular attention towards the the sexual character of the host in close connection with sex transformation. No paper, however, has till now been published concerning gonadectomy or gonadal transplantation of grasshoppers, so far as the author is aware. It was pointed out by the present experiments that no pronounced effect has been demonstrated on the secondary sexual characters of the host, when the testes were transplanted in female bodies. In the present paper, the problem in relation to the sexuality is put out of consideration, and a particular attention shall be given only to certain cytological phenomena involving the mitotic abnormalities of germ cells in the grafted testes. Some remarks on this respect will be made below.

As stated in the foregoing descriptions, many mitotic abnormalities connected with cell degeneration have taken place in the grafted testes of grasshoppers. They are represented by stickiness and coalescence of chromosomes, swelling of chromosomes, deformation of chromosomes, chromosome bridges and irregular separation of chromosomes at anaphase, unusual arrangement of chromosomes at metaphase, and occurrence of multi-nucleated cells. It is apparent that these abnormalities may disturb the normal course of development of the cells, and consequently they may constitute a cause or at least a morphological indication of cell degeneration. The present author has recently shown various abnormal mitotic figures, which closely resemble those obtained here, in an experiment of the germ cells of a grasshopper (*Podisma sapporensis*) carried out by treating the testes with colchicine solution. Momma (1950) has also demonstrated abnormalities of a similar nature in the germ cells of *P. sapporensis* after treatment with high temperatures. Furthermore very interesting and noticeable is the fact that Nakamura and Makino (1950) have reported cytological abnormalities, just the same as those observed in the present investigation, in experiments of artificial cryptorchism in rats carried out through elevation of the testis from the scrotum into the abdominal cavity.

There is a remarkable evidence indicating that the majority of the abnormal

figures which the above authors experimentally obtained strikingly resemble the mitotic abnormalities artificially obtained in plants by subjecting cells to high or low temperatures, by treating them with various chemicals or hypotonic or hypertonic media, or by exposing them to X-rays (Kuwada 1937, Shinke 1939, Sigenaga 1937, 1945). Very recently Kanô (1950) has again demonstrated mitotic abnormalities of similar kinds in germ cells of *P. sapporensis* by treating them with some hypotonic or hypertonic solutions.

According to Shinke (1939), there are two groups in the mitotic abnormalities which were caused by various artificial ways. The chromosome bridge, chromosome coalescence, pseudo-amitosis, di-diploid nucleus and failure of telophasic reconstruction are abnormalities which are obtainable by a mere treatment with hypertonic solutions, and their occurrence may be regarded as connected with dehydration that takes place in certain regions of the cell. The abnormalities such as chromosome scattering, irregular distribution at anaphase and formation of micro-nuclei have been regarded as abnormalities that take place in connection with hydration of the cell. Based on these facts, it is most probable that dehydration and hydration phenomena in the cell are in a close connection with the occurrence of many mitotic abnormalities.

In the paper dealing with experimental cryptorchism in the rat, Nakamura and Makino (1950) expressed their view thus: Generally speaking, it is very important that in the cell a definite water-relation should be maintained for the physiological performance of living phenomena, and thus, the hydration-dehydration theory of abnormal mitosis seems to be significant for the case of the cryptorchid testis too. They further stated that the normal environment of the testis may, due to an abdominal retention, be changed in regard to temperature or in some other physiological conditions, and that under such abnormal conditions, the water-relation in the cell may undergo changes to a more or less marked degree, and this may be followed by dehydration or hydration phenomena, resulting in the production of various mitotic abnormalities. Regarding the present case, it is a problem being in need of further investigation whether or not the mitotic abnormalities observed in the grafted testes can be accounted for as merely due to hydration or dehydration of the cell. But it seems probable to the author that a change in normal water distribution in the cell may be taken as a favourable basis of interpreting the cause of the mitotic abnormalities found in the present experiments too. Thus, the presumption may be allowed that the normal environment of testes may, by transplanting them in the body cavity of a different individual, be changed in regard to some physiological conditions, such as those in the supply of blood and nutrient, and that under such abnormal conditions, the water-relation in the cell would undergo considerable changes. The changes of water-relation in the cell may form, at least secondarily, a cause or causes for dehydration or hydration of the cell and as the result many mitotic abnormalities may be produced.

Summary

The present paper deals with histological and cytological investigations of testicular grafts implanted in the body of a female, carried out on some acridian grasshoppers, *Podisma sapporensis*, *Miramella mikado*, *Eiveneophilus longipennis* and *Oxya jezoensis*.

So far as the present observations are gone, the testicular transplantation into the body of a female individual brings about no modification of the sexual character of the host.

In the interspecific transplantation the grafted testes show mostly a considerable atrophy. In the intraspecific transplantation, the hosts permit generally the development of the grafts to a considerable extent, but the germ cells contained exhibit many abnormalities.

On the cause of these mitotic abnormalities occurred in the grafted testes, a tentative conclusion was drawn that, under some abnormal physiological conditions of the testis induced by means of transplantation, the normal water distribution in germ cells would be disturbed and this might be followed by dehydration or hydration phenomena resulting in the production of abnormalities of cell and nuclear divisions.

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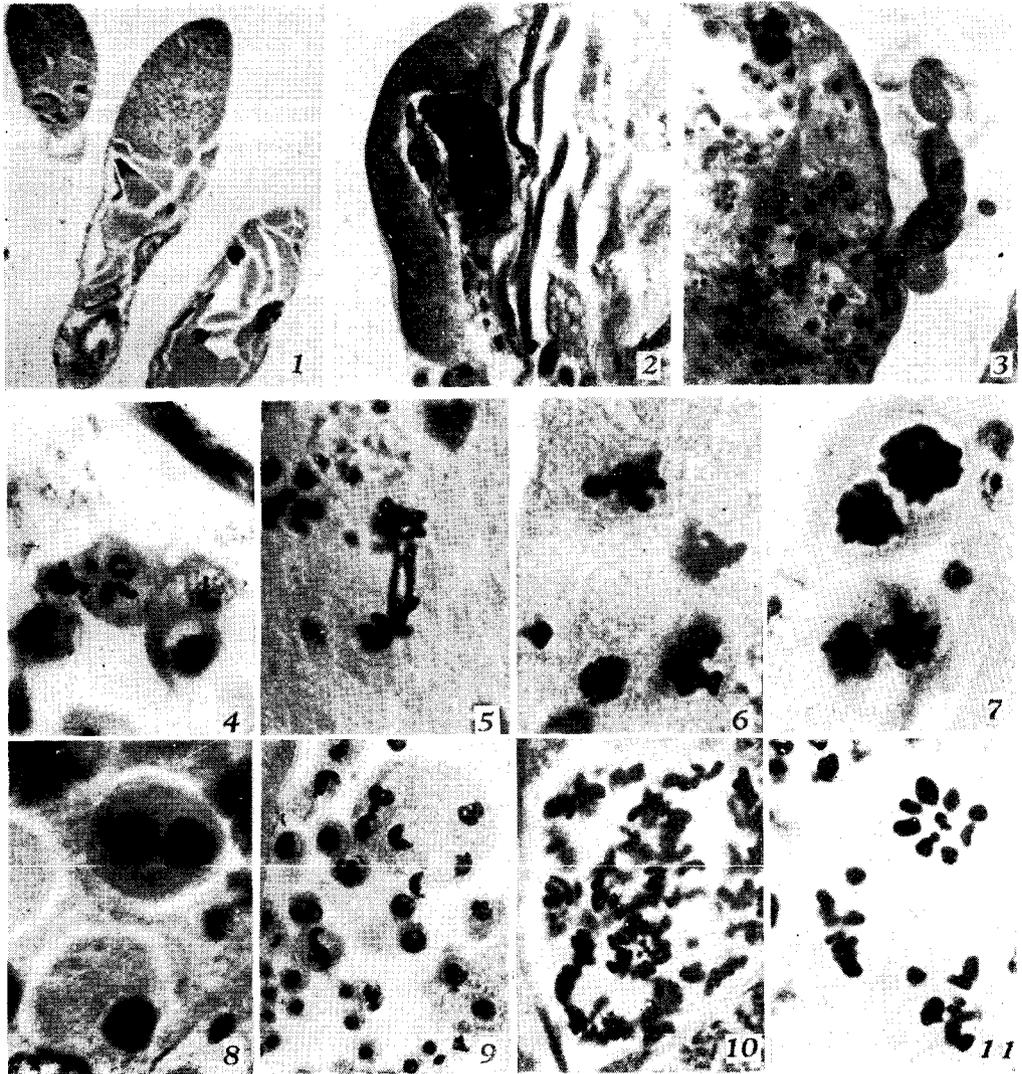
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Explanation of Plate IV

Photomicrographs indicated in Figs. 2, 3 and 4 were taken with "Leitz Makam" by the courtesy of Prof. S. Makino. Others were taken with "Canon camera" by the author. Fig. 1: $\times 50$. Figs. 2, 3 and 9: $\times 200$. Fig. 4: $\times 400$. Figs. 5-8, 10-11: $\times 1000$.

- Fig. 1. Testis-follicles of *Oxya jezoensis* implanted in male body, for 20 days.
- Fig. 2. Showing a remarkable thickening of the epithelial tissue of the testis-follicle of *Podisma sapporensis* located for 10 days in female body.
- Fig. 3. Striking outgrowth of the epithelium of testis-follicle, from the same material as Fig. 2.
- Fig. 4. Unequal distribution of chromosomes of *P. sapporensis*, in the first division in testis grafted for 10 days in female body.
- Fig. 5. The chromosome bridge appeared in the first division in testis of *Miramella mikado* left for 20 days in female body.
- Fig. 6. Chromosome clumping observed in the first division of testis of *O. jezoensis* grafted for 10 days in female body.
- Fig. 7. Bi-nucleated cells occurred in testis of *O. jezoensis* implanted for 20 days in female body.
- Figs. 8 and 9. Pycnotic aggregations of chromosomes, observed in the same material as Fig. 7.
- Figs. 10 and 11. Regular mitotic and meiotic divisions, occurred in the testis of *O. jezoensis* implanted for 30 days in female body.



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