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Observations on the Copulation of a Freshwater Planarian, *Polycelis sapporo*¹⁾

By

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(With 6 Text-figures and 3 Plates)

The copulatory behavior of planarians was first reported by von Baer in 1827. Since then several others have occasionally dealt with the copulation of both freshwater and marine triclads (*cf.* Hyman '25). A survey of the literature leads us to conclude that the number of copulants observed was extremely limited and the entire procedure of copulation was described in only a few cases. Very recently Jenkins and Brown ('64) were successful in taking careful note of the entire procedure of copulation in *Dugesia dorotocephala*, with some photographs showing various positions and types of behavior during copulation.

A freshwater planarian, *Polycelis sapporo*, is a common species and has been easily collected in Hokkaido. However, little is known about the life cycle and reproduction of this species. The author came to observe a number of copulants in the laboratory in the fall each year. In the present study will be offered a detailed description of the manner and the internal relations of the genital organs of the copulants.

Material and Method

The material used in the present study was a freshwater planarian, *Polycelis sapporo*, which was found in the small stream near the lodge (1050 m above sea level) at Mt. Muine, near Sapporo. Nearly mature worms were found during every month of the year both in the natural habitat and in the laboratory. The collection of these worms was performed on June 19th, July 21st and August 6th, 1963. At the time of collection, the temperatures of the water in the stream were 4.5, 8.0 and 9.5°C, respectively. The fresh worms thus collected were divided into several groups as stock to rear in glass vats with 4 litre of well water containing about 100 worms. The temperature was kept at $7 \pm 1.5^\circ\text{C}$ in a refrigerator. The animals were fed *ad libitum* on chicken spleen.

The manner of copulation was observed in a dark room by means of weak illumination for a few seconds at a time. An electric torch of about 85 lux, covered with red cellophane, was used. In order to take photographs of the copulatory activities in living worms, the Petri-dish containing the copulants was quickly but carefully transferred into the field of

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vision of the dissecting microscope, and then the dish was illuminated momentarily by means of a foot switch with 250 W Reflector-flood lamps. Prefocussing on the smooth bottom surface of the dish had already been obtained. The shutter speed was either 1/50 or 1/125 second.

For the anatomical observation of the genital organs in the copulant, several pairs were taken out for fixation. They were fixed in Bouin's or Zenker's fluid and were sectioned by the ordinary paraffin method. The serial sections were stained with Delafield's hematoxylin and eosin.

Results

Conditioning for the occurrence of copulation: The size-structure and the condition of the reproductive organs in two populations, collected on June 19th and July 21st, are summarised in Fig. 1 and table 1. As shown in the table, nearly all the worms measuring longer than 13 mm in body length have a copulatory organ

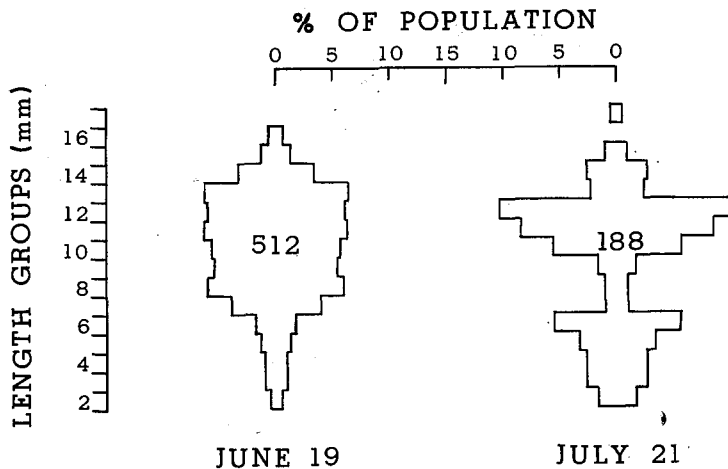


Fig. 1. Size-structure in Muine populations collected on June 19th and July 21st. Inset numbers refer to total population size.

Table 1. The condition of the reproductive organs in Muine population.

Date of collection	Body length of worm	Percent of worms having copulatory organ	Percent of worms having mature testes
June 19th	Longer than 13mm	98	100
	12-8mm	47	69
	Shorter than 7mm	0	0
July 21st	Longer than 13mm	92	100
	12-8mm	88	92
	Shorter than 7mm	0	0

and mature testes. A copulatory organ and mature testes are found also in a percentage of worms of the middle size, *i.e.* 8–12 mm in body length, but are never found in the worms shorter than 7 mm. The incidence of copulation is highest in the worms measuring 13 mm, and lowest in the worms measuring 11–12 mm and 14–16 mm.

In order to ascertain suitable conditions for the occurrence of copulation, a preliminary test was performed in the following way. The worms just after collection were divided into 4 groups consisting of about 100 worms each. They were reared in the following separate conditions:

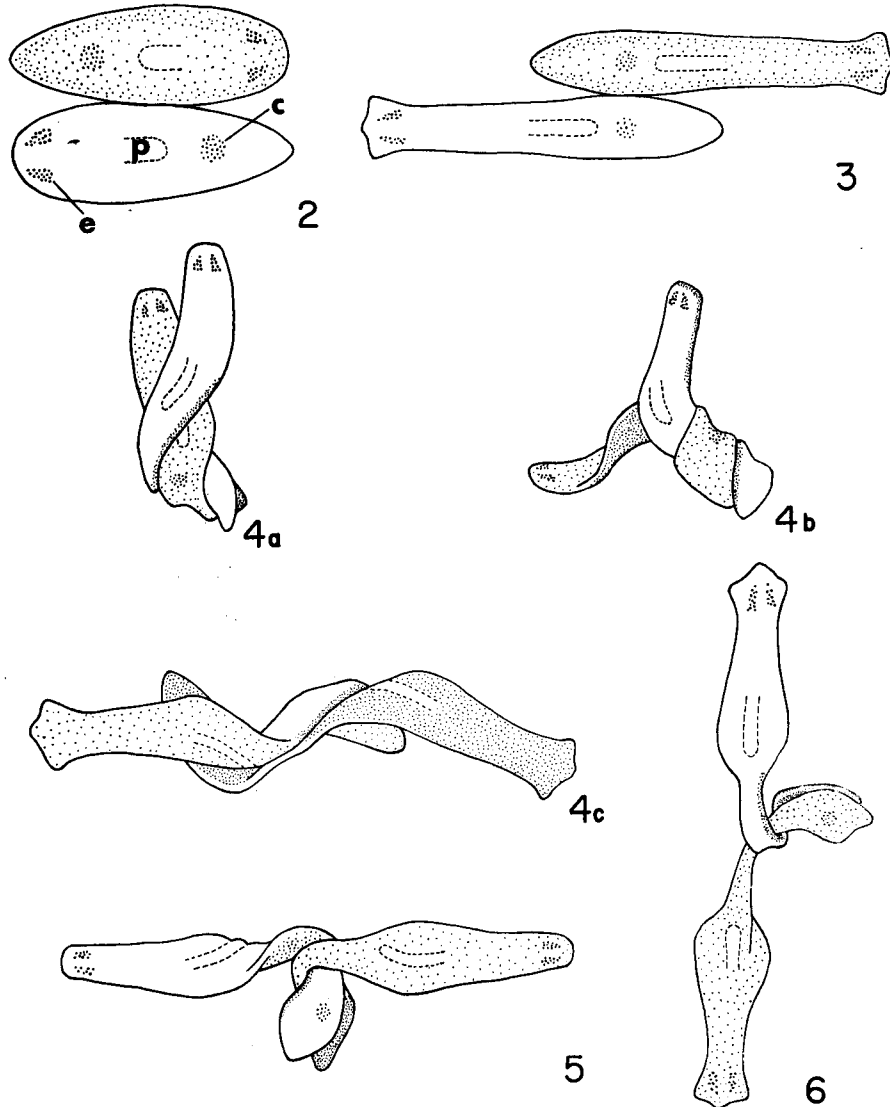
- Group A: dark condition in a vat containing 4 litre of well water in a refrigerator at $7 \pm 1.5^\circ\text{C}$.
- Group B: dark condition in a vat containing 4 litre of well water kept at $11 \pm 1^\circ\text{C}$.
- Group C: exposed to diffused light in a vat containing 4 litre of well water kept at $11 \pm 1^\circ\text{C}$.
- Group D: exposed to diffused light in a vat with running well water kept at $11 \pm 1^\circ\text{C}$.

After 50 days' rearing, 29 copulating pairs were noted in Group A, 20 pairs in Group B, 6 pairs in Group C and 5 pairs in Group D. Repeated examination of the worms derived from different batches gave similar results. That is, a considerable number of copulating pairs were noted in the glass vat in the dark room while only a limited number of the copulants were found in the diffused light. No significant difference was found between Groups A and B, and Groups C and D, respectively. Furthermore, copulation took place at any time of the day in Groups A and B. In Groups C and D, however, copulation occurred always either in the evening or at midnight. The author also observed natural copulation on three occasions. In these cases, the copulation always occurred in the dark condition, *i.e.* 2 cases in the evening and 1 case early in the morning. The results of the observations mentioned above indicate, therefore, that the worms prefer darkness for copulation.

The process of copulation: Based on the results described above, the observation on the copulatory process was carried out on the worms reared under dark conditions. Copulation in a total number of 83 pairs was found during July 13th to October 18th.

Usually copulation occurs in the following manner (*cf.* Figs. 2–6). When two worms wandering on the glass wall happen to come in contact, they stay in parallel with each other, their anterior ends being oriented in opposite directions and their heads lifted free from the substratum into the water. In this state, each worm shakes its head up and down for several minutes, and then shortly the worms are huddled up in an oval shape and become still. Within several minutes they begin to give each other quick little touches with the lateral margin at the level near the mouth. This action continues for about 30 minutes (Fig. 2). Then both worms

glide slowly forward for a short distance in opposite directions, continuing to move until the right or left lateral margin of the postpharyngeal region of one partner comes in touch with the margin of the same level of the other partner (Fig. 3). After



Figs. 2-6. Drawings showing successive phases of copulatory process. Detailed explanation in text. Fig. 4c, ventral view of the copulants in the same phase as Fig. 4a. c: copulatory organ, e: eye-band, p: pharynx.

a short interval of less than 20 seconds, the two worms lift their postpharyngeal regions free from the substratum and twist them so that both ventral surfaces of postpharyngeal regions come in firm contact (Figs. 4a-4c). The anterior ends of both copulants are firmly fixed to the substratum, oriented in the same direction (Fig. 4a). Then they become motionless, with the postpharyngeal regions remaining elevated from the substratum, and engaged very firmly. Nearly 1 hour after that, a marked dimple appears on the dorsal surface of the copulants in the region of the genital pore. After this state continues for 1 to 2 hours, the head regions begin to move slowly back and forth in different directions, while the engaged area remains closely connected. After a while the anterior ends of the copulants become oriented in precisely opposite directions, with the ventral surfaces of the posterior portion keeping in close contact and becoming elevated into the water at right angles to the substratum (Fig. 5). Then relaxation and lengthening occur in both anterior portions, and once more these portions become firmly fixed to the substratum. After 3 to 6 hours in this position, the copulants begin to separate by pulling away in opposite directions (Fig. 6).

Of 39 pairs measured exactly, the duration, *i.e.* the time from the beginning of firm connection to the end of separation, averaged 5.3 hours, with 9.5 hours being the maximum and 2.1 hours as the minimum. The sizes of both copulants were approximately the same in the majority, and the difference in the body length of each copulant was within 1 mm; while the largest difference of the body length observed was 2 mm.

Besides the above mentioned manner, some unusual cases were noticed at the time of firm connection. For instance, after firm connection the anterior portion of one partner begins to turn gradually around the point of connection like the hand of a watch and continues to turn until the angle between it and the corresponding portion of the other partner becomes an acute angle. Later these anterior portions move in opposite directions and the two copulants become positioned exactly opposite to each other.

The copulants in firm connection cannot be disturbed by another worm or by feeding. In other words, two copulants remain quite indifferent, even when an inquisitive third planarian crawls under or over them. Furthermore, if food is placed near them, they remain quiescent without turning about to take the food but the solitary worms to gather round the food. If a moderate flow of water is introduced during the firm connection, one partner crawls about with its anterior portion, dragging the other partner. The ventral surfaces of the posterior portions remain in close contact for several minutes, but finally the copulants draw away from each other.

Observations on the sectioned material: Several copulating pairs were fixed at the time of firm connection and the internal relations of genital organs were studied in sections. In the copulants the ovovitelline duct is completely formed with clearly lined epithelial cells (Fig. 12). The opening of the common ovovitelline duct

into the male antrum is also perfect (Fig. 13). The yolk gland is composed of extremely large cells which contain a number of granules stained deeply with eosin (Fig. 14). Further, there are a number of cement glands which stain deeply with eosin (Fig. 15). The ovaries contain a number of large spherical oocytes (Figs. 16-17). The most developed oocytes found in the ovary seem to be near the end of the growth period in the oogenesis. The testes are filled with a mass of intertwined spermatozoa in the internal cavity and with numerous spermatids at the peripheral zone. All these observations prove that the copulants are fully mature and of high sexual activity.

In the serial sections of the copulants, the eosinophilic adhesive cells are present in the ventral surface of the posterior portion which is in close contact with the ventral surface of the partner (Fig. 18). Near the genital pore, on the other hand, are found sticky secretions which stain deeply with hematoxylin (Figs. 13 and 22).

The genital pores of each copulant are greatly enlarged and the penis papilla is elongated and slender, and its tip reaches near to the opening of the bursal canal through the genital pore of the partner (Figs. 22-25). The tip forms a spherical mass due to the contraction of muscle fibers. In the lumen of the tip remain the eosinophilic granules secreted from the penis glands (Fig. 19). Further, masses of these granules are found in the bursal canal and anterior portion of the copulatory bursa of both copulants, and each mass encloses bundles of spermatozoa (Fig. 20). Since these granules are similar in their staining properties to the granules in the penis lumen of the partner, it is probable that the granules in the copulatory bursa of the copulant have derived from the penis of the partner. Further, the quantity of spermatozoa in the spermiducal vesicle of both copulants is smaller than in the solitary worm (Fig. 21). In the neck of the ovary, *i.e.* the beginning of the ovovitelline duct which is termed the seminal receptacle, there is a mass of spermatozoa (Fig. 12). In the copulants fixed 1 hour after the beginning of firm connection, *i.e.* at the phase when a marked dimple appears on the dorsal surface in the region of the genital pore, seminal fluid is found in each copulatory bursa. Because the seminal fluid is never found in the copulatory bursa of two copulants fixed before the appearance of the marked dimple, the seminal fluid found here seems to be ejaculated by the partner. In addition to this, it is noticeable that the spermatozoa reach the seminal receptacle 1/2 hour after they have been ejaculated into the copulatory bursa.

Discussion

Jenkins and Brown ('63, '64) maintain in *D. dorotocephala* that the worms are sexually mature every month of the year and the season of the year appears to have little effect on copulatory activity. This is inconsistent with the present material, in which it is shown that a number of pairs began copulation from the end of summer to the fall. The occurrence of copulation in a particular season of the

year has been reported also concerning some species of *Dugesia* (Burr '12). In the present material the deposition of cocoons follows 10 to 40 days after copulation. A considerable number of cocoons have been collected in laboratory. Although the worms in the season of copulation are fully mature sexually as observed in the sectioned material, the state of the reproductive organs during the remaining part of the year has not been thoroughly studied. The factors affecting the sexual maturity as well as the copulatory activity are the problems to be solved in the present material.

The present observations indicate that darkness is apparently more favorable than light for the occurrence of copulation. Exposure of the worms to light in the early phases of copulation frequently causes disturbance of copulation to varying degrees including considerable shortening of the duration of copulation or even the complete disturbance of the occurrence of copulation. The same observation that copulatory worms generally prefer darkness to daylight, has also been described by Burr ('12). In *D.* (= *Planaria*) *polychroa*, however, the copulating pairs were found also in the daytime and were hardly disturbed even when exposed to the sun. Furthermore, there are indications in *D. dorotocephala* that exposing the worms to light following a period of darkness is effective in instigating copulation (Jenkins & Brown '64). In the light of these facts, copulants of *Pol. sapporo* appear to be more sensitive to light than the other species.

The duration of copulation varies considerably according to several authors from a minute or less to 3 hours. Rather longer is required for the completion of copulation in the present material. The variation in the duration may partly be due to the limited number of observations made: in most cases the exact beginning of the act of copulation was not observed in the earlier works (cf. Jenkins & Brown '64). In addition the manner of copulation may differ according to the species studied. For example, no mating preliminaries of any kind were found in *D. dorotocephala* (Jenkins & Brown '64). In the present material, however, preliminary courting for about 1 hour is clearly detectable. Preliminary courting activities are then followed by the attitude reported by Budington ('24) and Jenkins and Brown ('64). In *Dugesia tigrina* (= *Planaria maculata*), a case has been described in which two worms mate with heads in the same direction. In *D. dorotocephala*, this attitude was found during the second stage, when the copulants were completely immobile and strongly engaged, with the greater part of the ventral surface in close contact. Since the posterior portions of the copulants in the present material are very firmly interlocked and each ventral surface is in close contact, it is possible to deduce that the ejaculation of spermatozoa takes place at this phase. The position of each copulant oriented in opposite directions at the end of copulation is similar to that commonly depicted in several planarian species (von Baer 1827, Dugès 1828, Hallez 1887, Kennel 1889, Wilhelmi '09, Burr '12).

It has been noted in some triclads that frequently one of the pair takes a more active part in the copulation, with the other remaining somewhat passive

(Wilhelmi '09, Hyman '25). In the present study, there was no evidence that one partner was "active" and the other "passive". This is supported by the observations on the sectioned material that the penis is inserted mutually into the bursal canal and that spermatozoa are ejaculated into the copulatory bursa. Further, it may be worthy of notice that the seminal receptacle at the beginning of the ovovitelline duct is filled with spermatozoa even right after the occurrence of copulation.

Summary

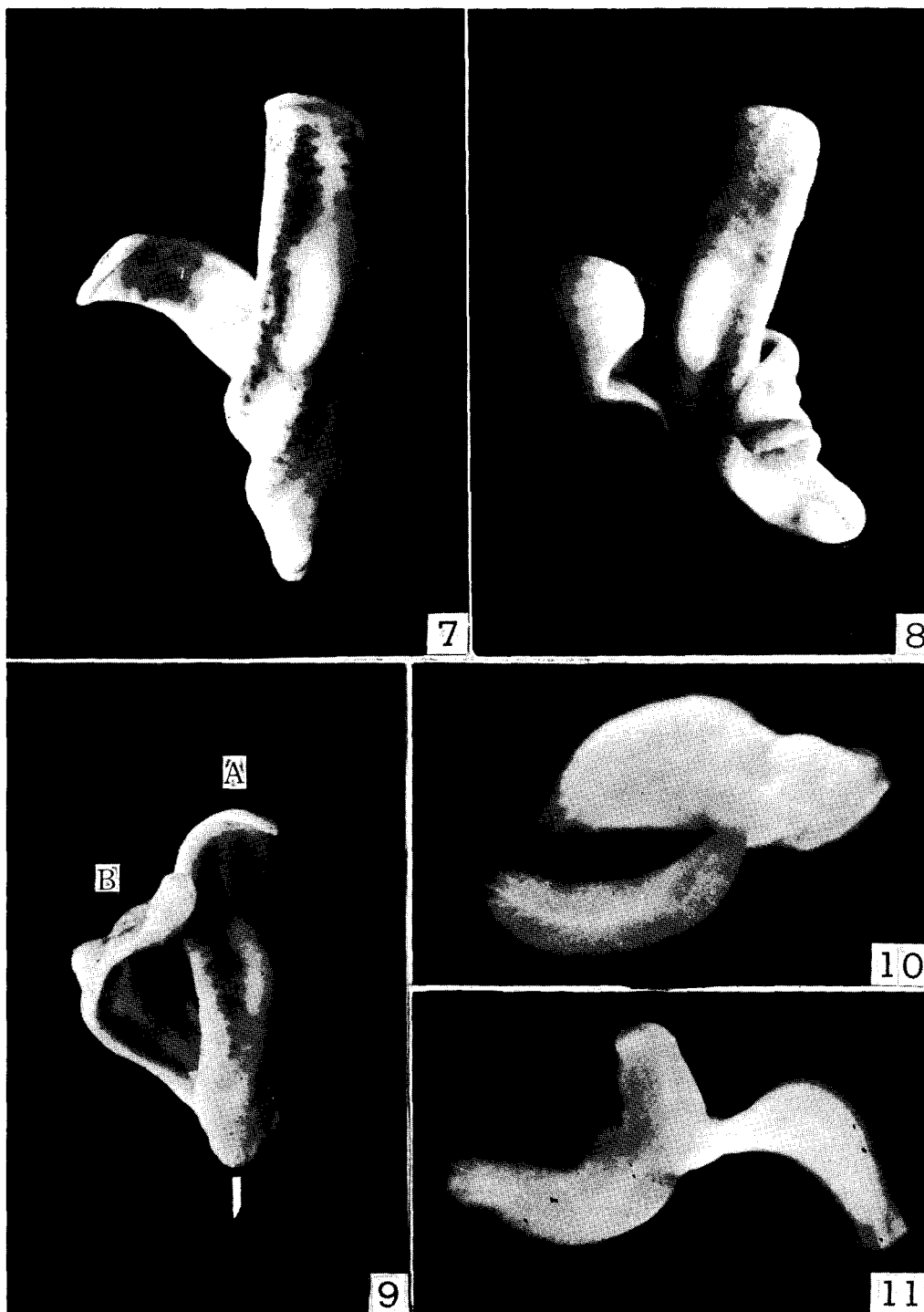
The copulation of a freshwater planarian, *Polycelis sapporo*, collected in the summer and reared in laboratory, was studied with the following results:

1. Darkness seems to be favorable for the copulants of this species.
2. At the time of copulation, the anterior ends of the animals are firmly fixed to the substratum; the ventral surfaces of the posterior portions are in close contact and elevated free from the substratum into the water.
3. A preliminary courtship activity is noted. There is no evidence of one partner being "active" and the other "passive".
4. The duration of copulation varies with each case from 2.1 hours to 9.5 hours.
5. Histological observations prove that the penis of the copulating pair is inserted mutually to the common antrum through the genital pore and the secreting granules of the penis glands with masses of spermatozoa are ejaculated mutually into the copulatory bursa. During copulation the spermatozoa have already passed through the ovovitelline duct to the seminal receptacles of both animals.

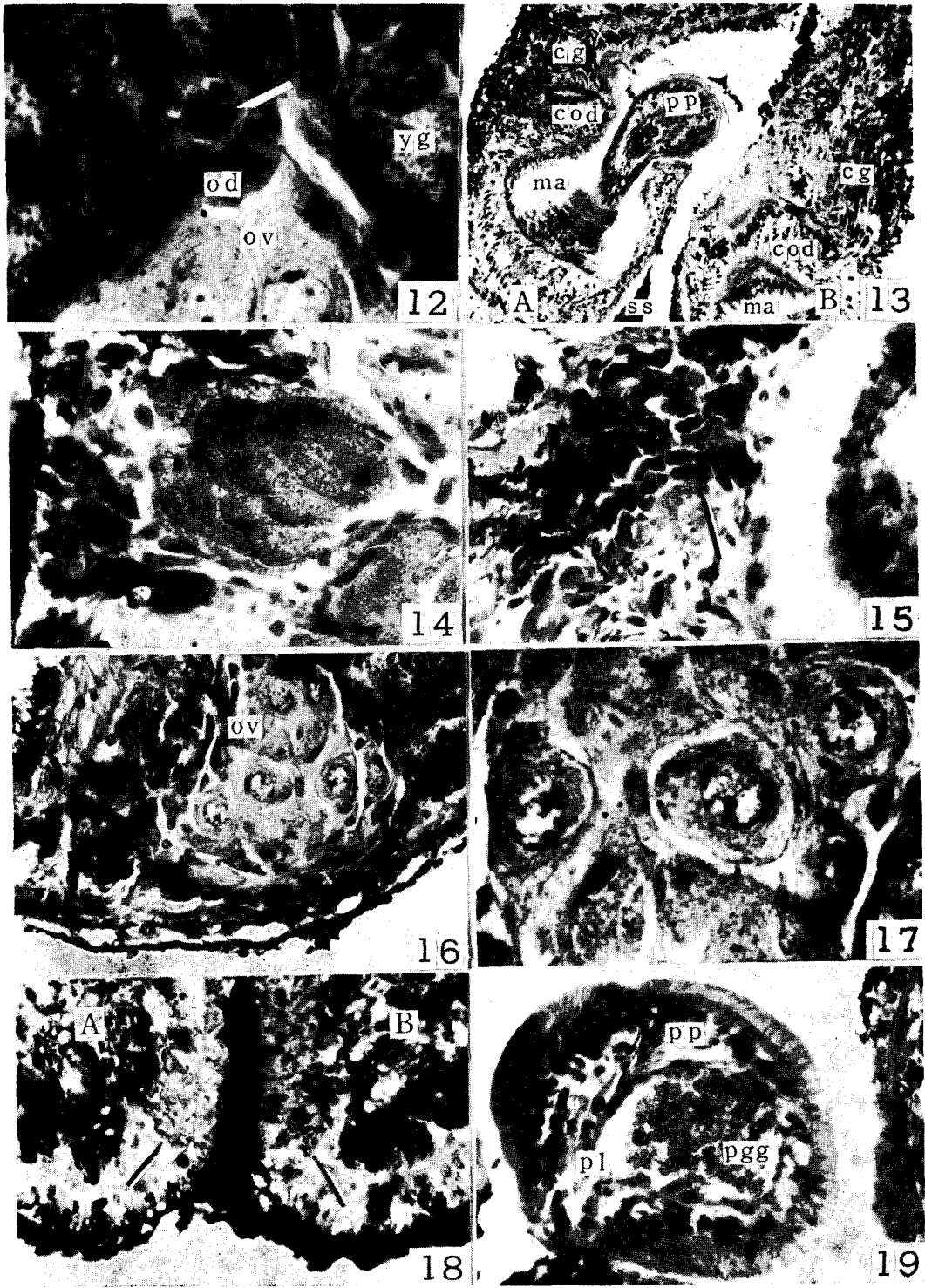
The author wishes to express his thanks to Professor A. Ichikawa for suggesting this study as well as for constant guidance in the course of the work. Thanks are also due to Dr. T.S. Yamamoto and Dr. Ch. Katagiri for their invaluable suggestions and encouragement, and to Dr. M. Kawakatsu for some valuable advices.

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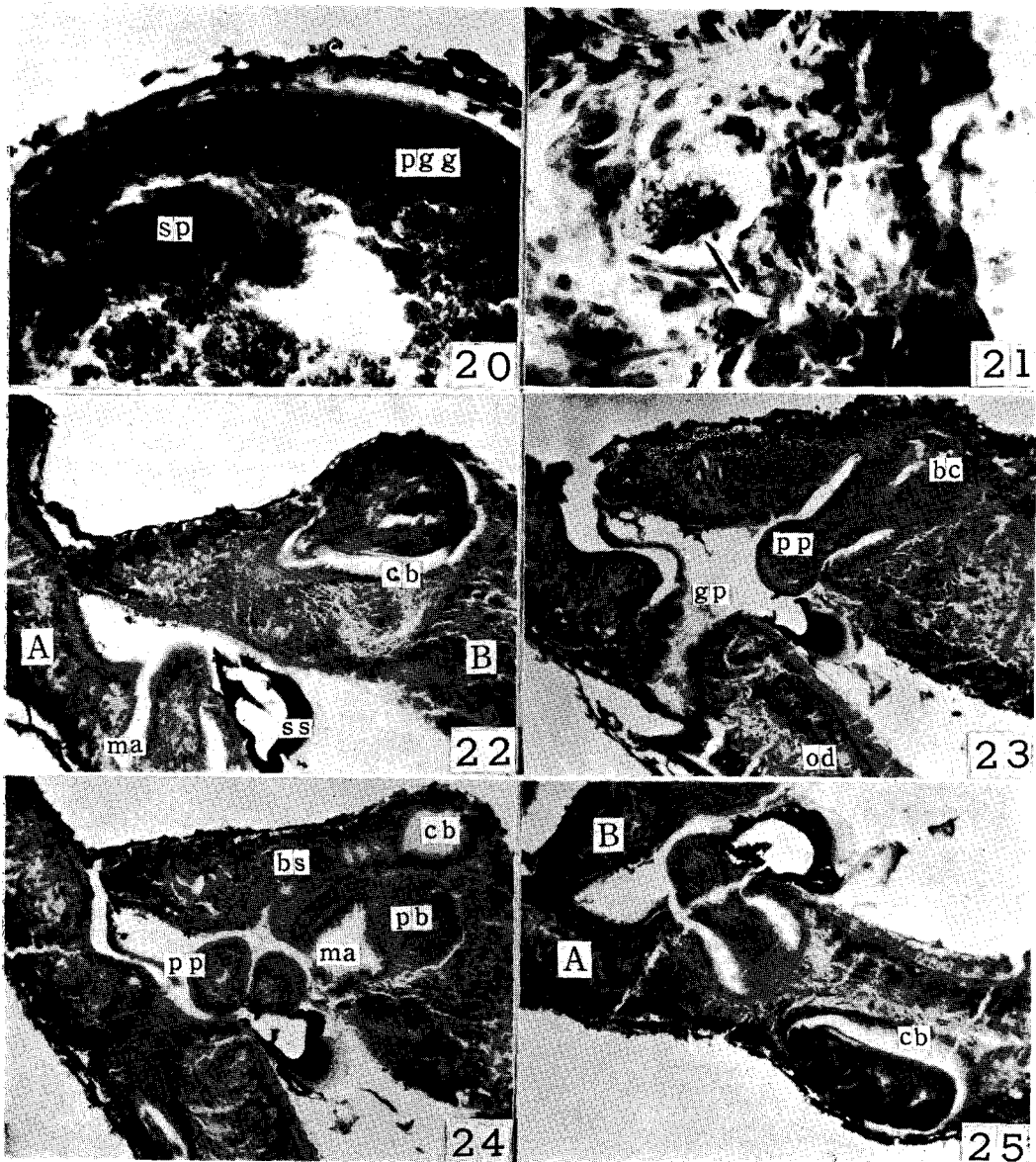
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Explanation of Plates XVIII-XX

Abbreviations: (bc) bulbar cavity, (bs) bursa stalk, (cb) copulatory bursa, (cg) cement gland, (cod) common ovovitelline duct, (gp) genital pore, (ma) male antrum, (od) ovovitelline duct, (ov) ovary, (pb) penis bulb, (pgg) granule secreted from penis gland, (pl) penis lumen, (pp) penis papilla, (sp) spermatozoa, (ss) sticky secretion, (yg) yolk gland.

Figs. 7-9. Copulating pairs photographed in fixed condition. ca. $\times 7$.

Figs. 10-11. Photographs of fresh worms in copula at the same phase as in Figs. 4a and 6. ca. $\times 6$.

Fig. 12. Section through ovary of copulant, showing a mass of spermatozoa (arrow) surrounded with epithelial cells of the seminal receptacle. ca. $\times 820$.

Fig. 13. Section through copulatory organ of copulants, showing the opening of common ovovitelline duct and the cement gland in worm A(A) and worm B(B). ca. $\times 80$.

Fig. 14. Sagittal section through yolk gland of copulant, showing eosinophilic granules in gland cells. ca. $\times 320$.

Fig. 15. Sagittal section through copulatory organ of copulant, showing cement gland (arrow). ca. $\times 320$.

Fig. 16. Sagittal section through ovary of copulant. Note large egg cells occupying most parts of the ovary. ca. $\times 320$.

Fig. 17. Section through ovary of copulant, showing large spherical egg cells. ca. $\times 820$.

Fig. 18. Section through posterior ends of copulants, worm A (A) and worm B (B). Note close contact with the ventral surfaces. Arrows indicate adhesive cells. ca. $\times 320$.

Figs. 19-25. Serial sections through the copulatory organs of copulants shown in Fig. 9, sectioned along the plane indicated by arrow in Fig. 9. A and B indicate the worms shown in Fig. 9, respectively. Figs. 19-21; ca. $\times 320$. Figs. 22-25; ca. $\times 80$. Note secreting granules in penis lumen at the tip of penis papilla of worm B in Fig. 19. The secreting granules and a mass of spermatozoa in copulatory bursa of worm A are seen in Fig. 20. Intertwined spermatozoa (arrow) in the spermiducal vesicle are found in Fig. 21.