Switching from Asexual to Sexual Reproduction in the Planarian Dugesia ryukyuensis: Change of the Fissiparous Capacity along with the Sexualizing Process

Authors: Kobayashi, Kazuya, and Hoshi, Motonori

Source: Zoological Science, 19(6) : 661-666

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.19.661

ZOOLOGICAL SCIENCE **19**: 661–666 (2002)

Switching from Asexual to Sexual Reproduction in the Planarian *Dugesia ryukyuensis*: Change of the Fissiparous Capacity along with the Sexualizing Process

Kazuya Kobayashi[1] and Motonori Hoshi[2*]

[1] *"Form and Function", PRESTO, Japan Science and Technology Corporation (JST)*
[2] *Center for Life Science and Technology, Keio University, Hiyoshi 3-14-1,*
Kouhoku-ku Yokohama 223-8522, Japan

ABSTRACT—Asexual worms of fissiparous strain of the planarian *Dugesia ryukyuensis* switch from asexual to sexual reproduction, if they are fed with sexually mature worms of *Bdellocephala brunnea*. This suggests that the sexually mature worms have a sexualizing substance(s) that induces the sexuality in the asexual worms. Here, we found by analysis of the sexualization that the cessation of the fission, namely their asexual reproduction, occurs immediately after the acquisition of sexuality. This result suggests that the downstream mechanisms induced by the putative sexualizing substance in *B. brunnea* become responsible for the cessation of fission. We also found that the decapitation triggers fission in the worms even after the acquisition of sexuality if they are not sexually mature, while the fully sexualized worms never fission even though they are decapitated. This result suggests that the cessation of fission takes place via at least two steps: (1) the mechanisms associated with the cephalic system; (2) other mechanisms independent of cephalic control.

Key words: planaria, asexual-sexual switch, sexualization, sexualizing substance, cessation of fission

INTRODUCTION

Many metazoans may reproduce both asexually and sexually from population to population within a species (Bell, 1982). They convert the reproductive modes presumably depending upon the environmental circumstances and/or the phase of life cycle. However, the mechanisms underlying the switching from the asexual to the sexual reproduction, and *vice versa*, remain unknown in metazoans.

Some planarians, especially in the genus *Dugesia*, comprise of at least three races with respect to the reproductive mode: asexual race, sexual race, and 'physiological race' (Jenkins, 1967). In the asexual race, worms reproduce by fission without sexual organs. In the sexual race, worms have hermaphroditic sexual organs, and copulate and then produce cocoons filled with several fertilized eggs. They are not likely to fission. In the physiological race, worms convert between asexual and sexual reproduction seasonally. In such planarians, it is well known that even the asexual race switches from asexual to sexual reproduction by being fed with sexually mature planarians (Grasso and Benazzi, 1973; Sakurai, 1981). Recently, we established an assay system for sexualization in the OH strain, an exclusively fissiparous strain, of *Dugesia ryukyuensis* by feeding with sexually mature worms of *Bdellocephala brunnea*, an exclusively oviparous species (Kobayashi *et al*., 1999). This experimental sexualization indicates the existence of the putative sexualizing substance in *B. brunnea*. We divided the process of the sexualization into five distinct stages by histological changes (Fig. 1) and found that the sexualization has a point-of-no-return. Before the point-of-no-return, the worms return to the asexual condition unless feeding on sexually mature worms of *B. brunnea* is maintained, whereas after the point-of-no-return, the worms are committed to the sexuality. In other words, the worms after the point-of-no-return no longer need feeding on the sexualizing substance contained in *B. brunnea* to be sexualized. In this experimental sexualization, it was observed that the fully sexualized worms do not fission. This suggests that cessation of fission, which is, in general, a significant event during the switching from asexual to sexual reproduction, may occur. So far, changes of asexual capacity (fissiparous capacity) in the sexualization were not yet reported, though the process of the development of sexual organs was described in detail. Although, at least, the sexualizing substance is directly responsible for the point-of-no-return, namely the acquisition

---

* Corresponding author: Tel. +81-45-566-1773;
FAX. +81-45-566-1448.
E-mail: hoshim@bio.keio.ac.jp

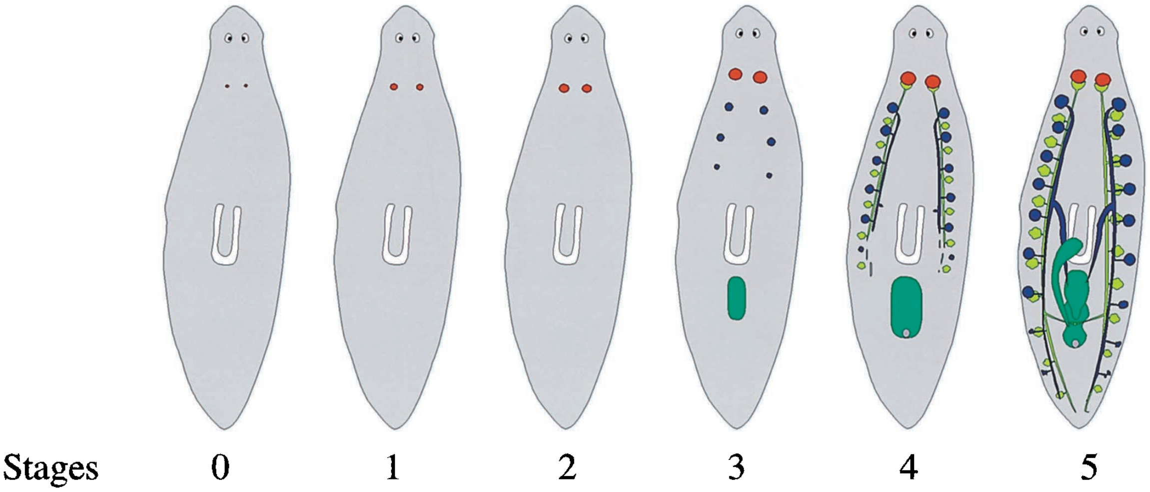

Fig. 1. Illustration of five distinct stages along with the sexualization. The development and topological position of reproductive organs are shown. Red region: ovary; blue region: testis and seminal duct; yellow region: yolk gland and oviduct; green region: a copulatory apparatus with a genital pore. It had been revealed by histological search that worms at stage 0 (the OH strain) have a pair of undeveloped ovaries. Briefly, at stage 1, the ovaries become larger enough to be externally apparent, yet neither oocytes nor other sexual organs are detectable. At stage 2, oocytes appear in the ovaries. At stage 3, the primordial testes and a copulatory apparatus emerge, and at stage 4, a genital pore in the copulatory apparatus become externally apparent, primordial yolk glands develop and spermatocytes appear in the testes. At stage 5, mature yolk glands are formed and many mature spermatozoa are detectable in the testes.

of sexuality, it is still obscure whether the relationship between the sexualizing substance and the cessation of fission is direct or indirect. In this study, we examined the fission along with the process of the sexualization, and estimated the correct period of the cessation of fission in contrast with the point-of-no-return. Finally, we discussed the relationship between the sexualizing substance and the cessation of fission.

MATERIALS AND METHODS

Animals

An exclusively fissiparous strain, the OH strain of the planarian *Dugesia ryukyuensis* presented by Dr. S. Ishida of Hirosaki University was maintained at 20°C in dechlorinated tap water by being fed with chicken liver. After starvation for two to three weeks, the worms were used as asexual recipients in a feeding experiment. Wild populations of *Bdellocephala brunnea*, an exclusively oviparous planaria, were collected in the vicinities of Yamagata City, Japan. They were used as the food to sexualize the worms of *D. ryukyuensis*.

The feeding experiment was performed as previously described (Kobayashi *et al*., 1999). Three groups of fifty worms of the OH strain were fed with the sexually mature worms of *B. brunnea* for six weeks. External observation was carried out under a binocular microscope every week to examine whether or not the recipients develop sexual organs (a pair of ovaries and a genital pore). Fission in the population was examined everyday. In case of fission in the recipients, the tail fragments were removed to keep the population density (five worms in about 30 ml of dechlorinated tap water), because the population density effects the process of sexualization (Kobayashi and Hoshi, unpublished data).

Regeneration experiment

The recipients were cut at the prepharyngeal level with a razor (Fig. 2). The head fragments were fixed with 10% formalin in phosphate-buffered saline (34 mM NaCl, 7 mM KCl, 2.5 mM Na_2HPO_4, 4.5 mM KH_2PO_4, pH 7.4). The fixed specimens were dehydrated through ethanol series, cleared in xylene and embedded in Paraplast Plus™ (Sherwood Medical, St. Louis, MO). The embedded specimens were cut into sections at 4 µm thickness and stained with hematoxylin and eosin. The stages of sexualization in the recipients were determined by the results of the histological examination of the head fragments according to Kobayashi *et al.* (1999) (Fig. 1).

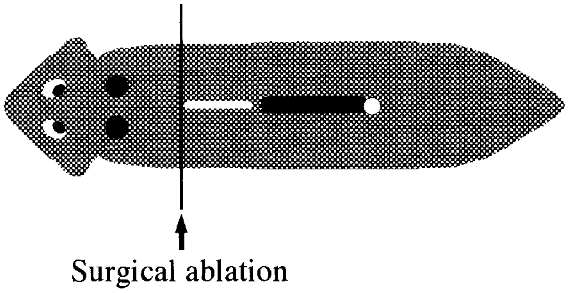

Fig. 2. Scheme of surgical ablation in the recipients. Surgical ablation was performed at the prepharyngeal level. The illustration of a planaria arranged to the anterior on the left. The two larger black dots represented a pair of ovaries; a white bar, a black bar and a white dot represented a pharynx, a copulatory apparatus and a genital pore, respectively. In case of the fully sexualized worms, the ovaries and the copulatory apparatus with a genital pore were apparent.

Additionally, each tail fragment was placed in a plastic dish of 35 mm in diameter and allowed to regenerate by being fed on chicken liver once a week. External observation was carried out every week for 6 months. When the tail regenerants developed sexual organs without fissioning, we regarded them as sexual worms. On the contrary, when they fissioned, we continued to maintain them. We regarded the fissioned offspring as asexual worms, when they fissioned further without developing sexual organs.

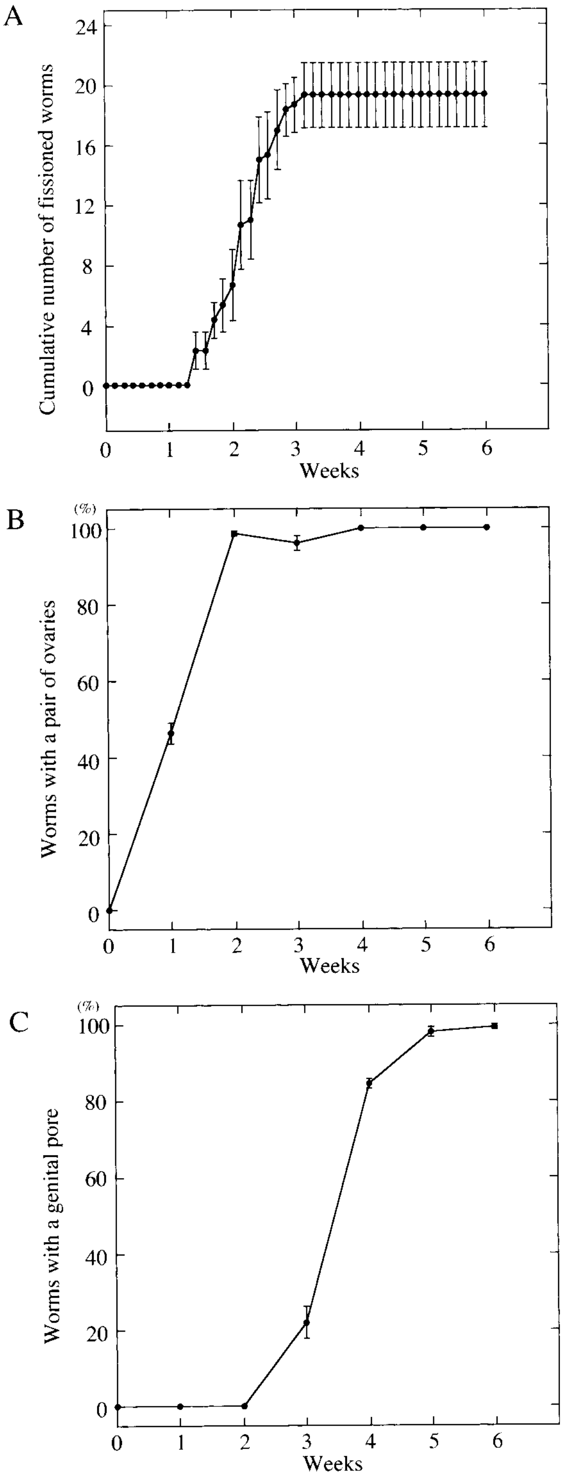

Fig. 3. The number of fissioned worms (**A**), the number of worms with a pair of ovaries (**B**) and the number of worms with a genital pore (**C**) along with the sexualization. Three groups of fifty worms of the OH strain were fed with the sexually mature worms of *B. brunnea* for six weeks. During the feeding experiment, second fission in the fissioned worms was not observed. Thus, total number of fission in the populations is expressed as the cumulative number of the fissioned worms. Means and standard errors (vertical bar) of the three groups were shown.

RESULTS

Fission in the recipients during sexualization

We divided the process of sexualization into five distinct stages by external observation and histological examination (Fig. 1, Kobayashi *et al.*, 1999). In this experiment, most worms at each week of feeding treatment approximately corresponded with stages 1 to 5 in order. Fig. 3A shows the number of fissioned worms along with the progression of sexualization. Worms fed on *B. brunnea* did not fission by Week 1, because the body size was not large enough to fission yet, and then they started to fission after about one week of feeding treatment. At almost the same time, a pair of ovaries were recognized externally in a half of the worms (Fig. 3B). From two to three weeks of the treatment, consequently, the worms with a pair of ovaries actively fissioned (Fig. 3A), though some of them developed a copulatory apparatus at the postpharyngeal region without fissioning. A few worms with copulatory apparatus, as well as a pair of ovaries fissioned. After three weeks of the treatment, fission was not observed (Fig. 3A). The worms that had not fissioned opened a genital pore on the ventral at Week 3 (Fig. 3C). Finally, the worms that had experienced fission also developed a copulatory apparatus and a genital pore after Week 4.

Regeneration experiment

In order to examine the fissiparous capacity and the degree of the sexualization in the recipients, we carried out the regeneration experiment as described in MATERIALS AND METHODS. Table 1 summarizes the fissiparous capacity of the recipients corresponding with the stages of sexualization (Fig. 1; Kobayashi *et al.*, 1999). In the case of the worms at stages 0–4, all the decapitated worms started to fission within ten days after surgery. The appearance of sexual offspring in the decapitated worms at stages 3 and 4

Table 1. The degree of sexualization and the fissiparous capacity in the recipients

Stages of sexualization[a]	Number of recipients	Number of fissioned recipients (%)
Stage 0[b]	15	15 (100)
Stage 1	9	9 (100)
Stage 2	11	11 (100)
Stage 3	14	14 (100)
Stage 4	11	11 (100)
Stage 5	15	0 (0)[c]

Sixty worms of the OH strain were fed with the sexually mature worms of *B. brunnea*. During 6 weeks of the feeding treatment, appropriate recipients were used in the regeneration experiment as described in MATERIALS AND METHODS.

[a] Explanation of the stages of sexualization was referred in Fig. 2.

[b] The recipients fed with chicken liver for six weeks were used as a control.

[c] Worms at stage 5, namely fully sexualized worms, did not fission, even though they were decapitated.

Table 2. The reproductive mode of the offspring from the decapitated recipients[a]

Stages of sexualization	Number of decapitated recipients	Average number of fission in decapitated recipients	Total number of offspring[b]	Reproductive mode of offspring: Number of sexual offspring(%)[c]	Reproductive mode of offspring: Number of asexual offspring(%)[d]
Stage 0	15	10.4	171	0 (0.0)	152 (100.0)
Stage 1	9	10.7	105	0 (0.0)	96 (100.0)
Stage 2	11	10.4	125	0 (0.0)	101 (100.0)
Stage 3	14	6.4	104	27 (30.3)	62 (69.7)
Stage 4	11	2.5	38	25 (75.8)	8 (24.2)
Stage 5	15	0.0	15	15 (100.0)	0 (0.0)

[a] The data was obtained from the recipients described in Table 1.

[b, c, and d] Total number of offspring is not identical with the sum of the number of asexual and sexual offspring, since the offspring in course of regeneration were not counted after 6 months of the regeneration experiment.

was followed by dramatic decrease in the frequency of fission (Table 2; as described below in detail). On the other hand, the decapitated worms at stage 5 never fissioned.

As a result of these cycles of fission, many offspring were obtained. We also examined their reproductive mode (Table 2). All the offspring at stages 0–2 became asexual. Both asexual and sexual offspring were observed in the decapitated worms at stages 3 and 4. The decapitated worms at stage 5 became sexual. When the decapitated worms at stages 3 and 4 fissioned, the head offspring always became sexual. However, the tail offspring followed quite a different fate along with the process of sexualization (Fig. 4): in type 1, the tail offspring produced two sexual worms in the same manner as the head offspring in the first cycle of the asexual reproduction, though it produced an asexual worm after two cycles of the asexual reproduction; in type 2, after a cycle of the asexual reproduction in the tail offspring, the head and tail regenerants became sexual and asexual worms respectively; in type 3, the tail offspring became asexual; in type 4, although the tail offspring underwent a cycle of the asexual reproduction, both the head and the tail regenerants became sexual; in type 5, the tail offspring became sexual. The decapitated worms at stage 3 followed the fate of types 1–3, while those at stage 4 followed mainly the fate of types 4 and 5, though a few of them followed the fate of types 2 and 3 (Table 3).

DISCUSSION

Fissiparous capacity during sexualization

Mostly, freshwater planarians (triclads) can reproduce asexually without sexual organs (Pearse *et al*., 1987). In such planarians, some populations may alternate between asexual and sexual reproduction (Curtis, 1902; Hyman, 1939; Jenkins, 1967). They cannot seem to fission, namely the asexual reproduction, whenever they keep a sexual state; that is, they not only develop hermaphroditic sexual organs but also cease to fission, when they switch from asexual to sexual reproduction (sexualization). However, the cessation of fission, a significant event in sexualization, is not described in detail.

We established an assay system for sexualization in the OH strain of *Dugesia ryukyuensis* by feeding with the putative sexualizing substance contained in *Bdellocephala brunnea* and divided the process of the sexualization into five distinct stages by histological changes (Fig. 1) (Kobayashi *et al*., 1999). The experimental sexualization has a point-of-no-return between stages 2 and 3. The worms at stages 3 onward no longer need feeding on *B. brunnea* to maintain the sexuality. We first examined fissioned worms along with the process of sexualization. External observation revealed that worms developing a pair of ovaries can fission, then they seem to cease the fission around the time they differentiate copulatory apparatus, and eventually they never fission after the differentiation of a genital pore (Fig. 3). Appearance of genital pore indicates that the worms were sexualized by stage 4. Thus, the cessation of fission is performed immediately after the point-of-no-return under the experimental conditions. We also observed that the worms after the point-of-no-return were fully sexualized without fissioning, even if feeding on *B. brunnea* was stopped (data not shown). These results suggest that, after the point-of-no-return, downstream mechanisms induced by the putative sexualizing substance become responsible for the cessation of fission. In other words, the sexualizing substance contained in *B. brunnea* would not be directly responsible for the cessation of fission, since the worms before the point-of-no-return are able to fission in spite of the progression of the sexualization. Grasso and Benazzi (1973) suggested that the putative sexualizing substance in sexual specimens serves to cease fission before the differentiation of sexual organs. The discrepancy between the two suggestions may be attributed to the difference in the experimental conditions.

Fissiparous capacity after decapitation

In order to estimate the fissiparous capacity of the recipients intensively and extensively, we carried out decapitation of the recipients by surgical ablation, because decapitation of asexual planarians facilitates the fission,

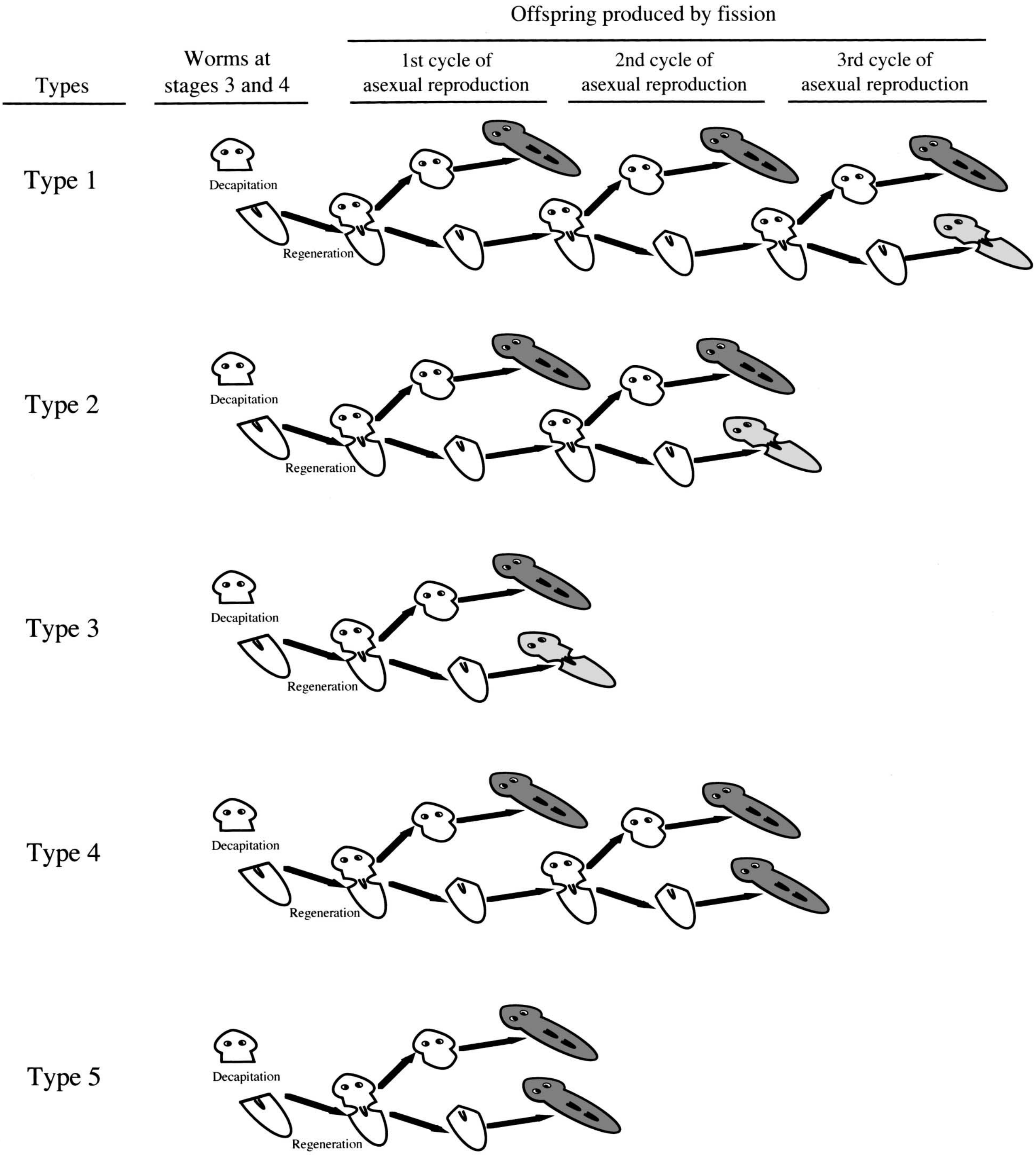

Fig. 4. Illustration of the different fates in decapitated worms at stages 3 and 4. Planarian illustrations painted with dark gray represented sexual offspring that developed full sexual organs without fissioning. Planarian illustrations painted with light gray represented asexual offspring that continued to fission without developing the sexual organs.

Table 3. Number in each type of the decapitated recipients*

Stages of sexualization	Number of decapitated recipients	Types of fate in decapitated worms shown in Fig. 4				
		Type 1	Type 2	Type 3	Type 4	Type 5
Stage 3	14	2	7	5	0	0
Stage 4	11	0	1	1	6	3

* The data was obtained from the recipients described in Table 1.

suggesting that the cephalic system (brain) controls fission (Best *et al.*, 1969; Best *et al.*, 1975). The decapitation induced fission in all the worms at stages 0–4, while it did not in those at stage 5 (Table 1). Interestingly, the decapitated worms at stages 3 and 4 fissioned, though the intact worms did not. This suggests that mechanisms responsible for the cessation of fission, which are induced by the sexualizing substance in *B. brunnea*, are associated with the cephalic system. On the contrary, in the worms after stage 5, namely, fully sexualized worms, other mechanisms independent of cephalic control may accomplish the cessation of fission, because the worms at stage 5, even decapitated ones, never fissioned.

Change of reproductive mode during cycles of fission

As a result of the regeneration experiment, many offspring were obtained by fission from the decapitated worms. The decapitated worms at stages 1 and 2 produced only asexual offspring, while those at stages 3 onward always produced sexual ones in spite of the lack of the putative sexualizing substance in *B. brunnea* (Table 2). This result supports the point-of-no-return between stages 2 and 3 (see described above; Kobayashi *et al.*, 1999).

We also found that the decapitated worms at stages 3 and 4 followed five distinct fates (Fig. 4, Table 3). During several cycles of fission, the head offspring always became sexual, while tail ones produced asexual offspring (Fig. 4; Types 1–3). In the decapitated worms at stage 4, almost all the offspring became sexual, though they underwent a few cycles of fission (Fig. 4; Types 4 and 5). It is notable that frequency of fission and appearance of asexual offspring dramatically decreased after the point-of-no-return (Table 2). Additionally, all the head fragments regenerated to become sexual without fissioning, if worms at stages 3 onward in which a copulatory apparatus became externally visible were cut as described in Fig. 2 (data not shown). This suggests that, within a planarian body, the acquisition of sexuality first takes place, and then the region unable to fission is committed from head toward tail gradually. In this paper, we conclude that the cessation of fission is determined after the acquisition of sexuality in the individual as well as at tissue level.

ACKNOWLEDGMENTS

We thank Dr. Sachiko Ishida, of Hirosaki University for her kind gift of the OH strain. Our thanks also go to Miss Karen Charman for her critical reading of the manuscript, and to Miss Yukako Shirato for her invaluable assistance in collecting animals. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 100082) to MH from the Ministry of Science, Culture, Sports and Education, Japan.

REFERENCES

Best JB, Goodman AB, Pigeon A (1969) Fissioning in planarians: Control by the brain. Science 164: 565–566

Best JB, Abelein M, Kreutzer E, Pigon P (1975) Cephalic mechanism for social control of fissioning in planarians III: Central nervous system centers of facilitation and inhibition. J Comp Physiol Psychol 89: 923–932

Bell G (1982) The paradox of sexuality. In “The masterpiece of nature: The evolution and genetics of sexuality” University of California Press, Los Angeles, pp 19–78

Curtis WC (1902) The life history, the normal fission and the reproductive organs of *Planaria maculata*. Proc Boston Soc Nat Hist 30: 515–559

Grasso M, Benazzi M (1973) Genetic and physiologic control of fissioning and sexuality in planarians. J Embryol Exp Morphol 30: 317–328

Hyman LH (1939) North American triclad Turbellaria. IX. The priority of Dugesia Girard 1850 over Euplanaria Hesse 1897 with notes on American species of Dugesia. Trans Amer Micros Soc 58: 264–275

Jenkins MM (1967) Aspects of planarian biology and behavior. In “Chemistry of Learning” Eds by WC Corning, SC Ratner. Plenum Press, New York, pp 117–143

Kobayashi K, Koyanagi R, Matsumoto M, Cabrera JP, Hoshi M (1999) Switching from asexual to sexual reproduction in the planarian *Dugesia ryukyuensis*: Bioassay system and basic description of sexualizing process. Zool Sci 16: 291–298

Pearse V, Pearse J, Buchsbaum M, Buchsbaum, R (1987) Flatworm Body Plan: Bilateral symmetry, Three layers of cells, Organ-system level of construction. Regeneration. In “Living Invertebrates” The Boxwood Press, Pacific Grove, California, pp 204–221

Sakurai T (1981) Sexual induction by feeding in an asexual strain of the freshwater planarian, *Dugesia japonica japonica*. Annot Zool Jap 54: 103–112

(Received September 28, 2001 / Accepted January 15, 2002)