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A Spontaneous Hermaphrodite of the Japanese Eel, *Anguilla japonica*, and Its Artificial Maturation

Hiroya Takahashi and Yoshio Sugimoto

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Abstract A spontaneous hermaphrodite was found out among a group of silver females of the Japanese eel, *Anguilla japonica*, collected in Aomori Prefecture, Japan. The hermaphroditic gonad was predominantly ovarian in anatomical and histological aspects, but was provided with numerous, discrete masses of testicular tissue of normal histoarchitecture which were localized along the distal edge of the gonad. The hermaphrodite was subjected to weekly injections of HCG followed by salmon pituitaries in an attempt to induce sexual maturation of the gonad. Successive biopsies of the gonad carried out before and during the hormonal treatment confirmed that both the testicular and the ovarian portions of the hermaphroditic gonad could rapidly develop into full maturation in response to the exogenous gonadotropins.

A great variety of intersexuality has so far been recorded to occur in various gonochoristic species of teleosts (reviewed by Atz, 1964). Among Anguilliformes, the European eel, *Anguilla anguilla*, is known to exhibit a juvenile hermaphroditism at the elver stage (Kuhlmann, 1975). So far as we know, however, no report has hitherto dealt with the occurrence of spontaneous intersexuality in the Japanese eel, *Anguilla japonica*, except for an occasional appearance of testis-ova in the testis of young eels (Satoh et al., 1962).

During the course of the studies on the induction of artificial maturation of silver females of the Japanese eel, we found out an eel with hermaphroditic gonads by exploratory laparotomy, and tried to stimulate the development of the hermaphroditic gonad by successive administrations of gonadotropic substances. In this paper, anatomical and histological characteristics of the hermaphroditic gonad of the eel are described together with the process of maturation of the gonad influenced by hormonal treatments.

Material and methods

The hermaphroditic eel was discovered in about 60 silver females of the Japanese eel, *Anguilla japonica*, which were collected in rivers around Hiranuma, Aomori Prefecture, Japan, in autumn 1976. They were transported to the laboratory and then acclimated stepwise to sea water. They were kept, without feeding, in concrete tanks containing sea water circulated with aeration and regulated at about 18°C under natural day length. After being marked individually by tagging, a group of females was subjected, beginning from early November 1976, to intramuscular injections of 100 IU human chorionic gonadotropin (HCG: Gonatropin, Teikoku Hormone Mfg. Co., Tokyo) per 100 g body weight once a week for 5 weeks and then 2 mg powder of acetone-dried chum salmon pituitaries per 100 g body weight once a week until up to full ovarian maturation.

At the beginning and the end of the HCG treatments, and just before each weekly injection of pituitaries, small pieces of gonads of these fish were sampled by laparotomy so as to explore histologically the advancement of ovarian maturation in individual fish. At the first laparotomy prior to the HCG treatment, a female, marked as 5-RY, was disclosed to have gonads with peculiar hermaphroditic conditions, so that it was possible to follow up the developmental process of the hermaphroditic gonad histologically by the aid of successive laparotomies during the period of the artificial treatments. The fish 5-RY was autopsied 5 days after the 3rd injection of pituitary powder, on 25 December 1976, for thorough investigations of the hermaphroditic condition.

Pieces of the biopsied gonads, and those taken at autopsy as well, were preserved in Bouin's fluid. Paraffin sections of the specimens were cut at 8～10 μ in thickness and stained with De-

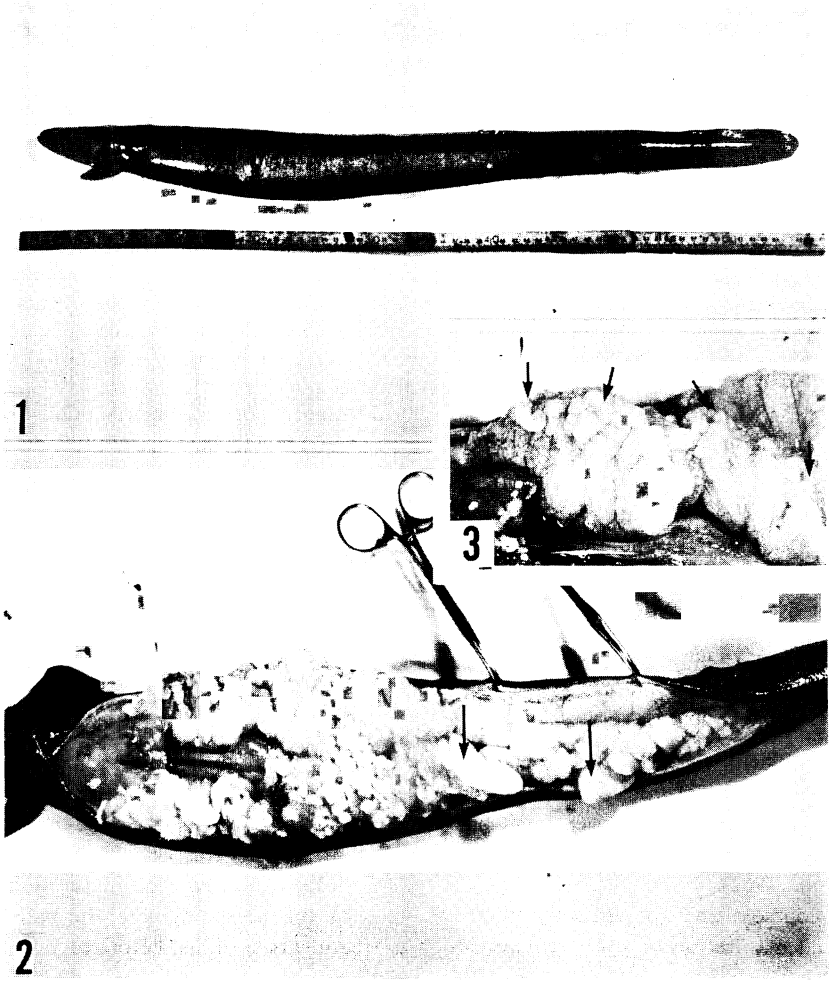


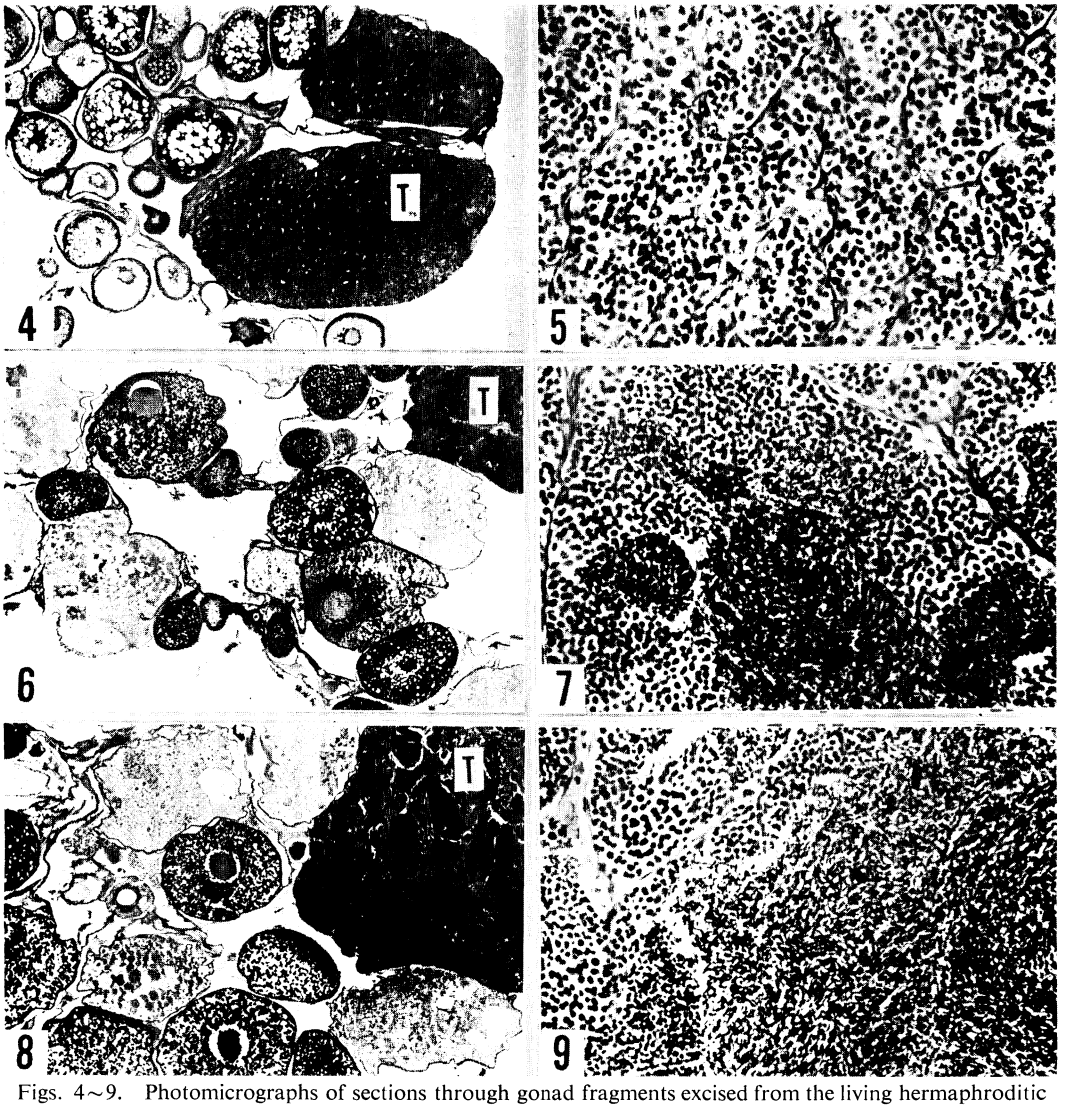
Fig. 1. Lateral view of the hermaphroditic eel, 65.9 cm in total length.
Fig. 2. Ventral view of the dissected fish, showing gross aspect of hermaphroditic gonads. Arrows indicate large testicular bodies existing in the caudal region of the gonad.
Fig. 3. Enlarged view of a part of the left hermaphroditic gonad, revealing testicular bodies (arrows) fringing the ovarian portion of the gonad.

lafield's hematoxylin and eosin for microscopic examinations. Developmental stages of oocytes were determined according to the description by Yamamoto et al. (1974).

Observations

The hermaphrodite, marked as 5-RY, measured 530 g in body weight and 65.9 cm in total length at the start of treatment. At autopsy, the fish increased in body weight to 620 g and its abdomen was observed to be slightly swollen, due to advancing maturation of the gonad (Fig. 1). The gonads, weighing 165 g in total, consisted predominantly of ovarian tissue which was quite similar in external aspects to that found in other female eels treated similarly with gonadotropins. However, the distal edges of the ovarian lamellae were fringed, along the whole length of the ovaries on both sides, by separate, small whitish bodies of various sizes and shapes (Fig. 2). It was confirmed by histological examinations that the whitish bodies were composed of testicular tissues which were normal in their histoarchitecture.

Many of the testicular bodies were roundish in shape and 5～7 mm in size, but a few of them



Figs. 4～9. Photomicrographs of sections through gonad fragments excised from the living hermaphroditic eel at the start of HCG treatment (Figs. 4 and 5), a week after the 5th injection of HCG (Figs. 6 and 7), and a week after the 2nd injection of salmon pituitaries (Figs. 8 and 9). Figs. 5, 7 and 9 demonstrate enlarged views of testicular portions (T) of the respective samples of the gonad. Figs. 4, 6 and 8, ×28; Figs. 5, 7 and 9, ×280.

existing caudal to the region of the urogenital pore were notably larger, the largest measuring 2.6×1.8×0.9 cm and weighing 2.8 g. Testicular bodies were distributed restrictedly on the distal free edges of ovarian lamellae and scarcely any of them occurred near the hilus of ovaries (Fig. 3), thus lacking entirely in the anatomically detectable sperm duct system. No essential difference was observed in the distribution pattern of testicular bodies between the right and the left gonad.

In histological preparations of the gonad biopsied before the hormonal treatment was initiated, masses of testicular tissue were found attached intimately to ovarian tissue, but were demarcated distinctly from the latter by thin layers of connective tissue in all cases observed (Fig. 4). In the ovarian portion of the hermaphroditic gonad, developing oocytes of 250～390 μ in size at the primary yolk stage and

those of 150～250 μ at the oil drop stage were the main germinal constituents. The testicular portion comprized young seminal lobules displaying an arrangement of a normal pattern, in which many cysts of spermatogonia together with some cysts of primary spermatocytes were present surrounding the narrow empty lumina (Fig. 5). The spermatogonia were seen frequently to be undergoing mitotic divisions.

Remarkable development of the hermaphroditic gonad was brought about by the treatment with HCG. In ovarian portion of the gonad biopsied following 5 weekly injections of HCG, there were many large oocytes exceeding about 600 μ in size and arriving at final stages of vitellogenesis, viz. the tertiary yolk and the migratory nucleus stage, along with younger ones of 400～600 μ in size at the secondary yolk stage (Fig. 6). The state of ovarian maturation extraordinarily surpassed that found in other treated females biopsied at the same time; in ovaries of the latter the most advanced oocytes were at most 250～300 μ in size and remained still at the primary yolk stage. It was remarked further that, in the hermaphroditic gonad, a striking variation occurred in the size and the developmental degree of oocytes existing in the same region of the ovarian portion.

Testicular portion of the hermaphroditic gonad was also promoted to maturation by the treatment with HCG, becoming to be provided with germ cell cysts at various stages of active spermatogenesis along the wall of seminal lobules and with ripe spermatozoa packing the central lumina of the lobules (Fig. 7). By the administration of salmon pituitaries which was performed successively following the HCG treatment, testicular portion of the hermaphroditic gonad came to mature nearly completely with a large quantity of spermatozoa in expanded lumina of seminal lobules a week after the 2nd injection of pituitaries (Fig. 9).

The maturation of ovarian portion of the hermaphroditic gonad also seemed to be advanced further by the pituitary administrations. In histological preparations of the gonad examined by biopsies just before the 2nd and the 3rd injection of pituitaries, however, ovarian tissue exhibited a similar condition of oocyte maturation to that observed in the ovarian portion just before the 1st injection (Fig. 8, compare with Fig. 6): oocytes constituting the ovarian tissue still ranged in their developmental stages from the secondary yolk to the migratory nucleus stage, though some of the large oocytes were seen to be in the process of degeneration especially in the case of the last biopsy.

The ovarian maturation induced and acceler-

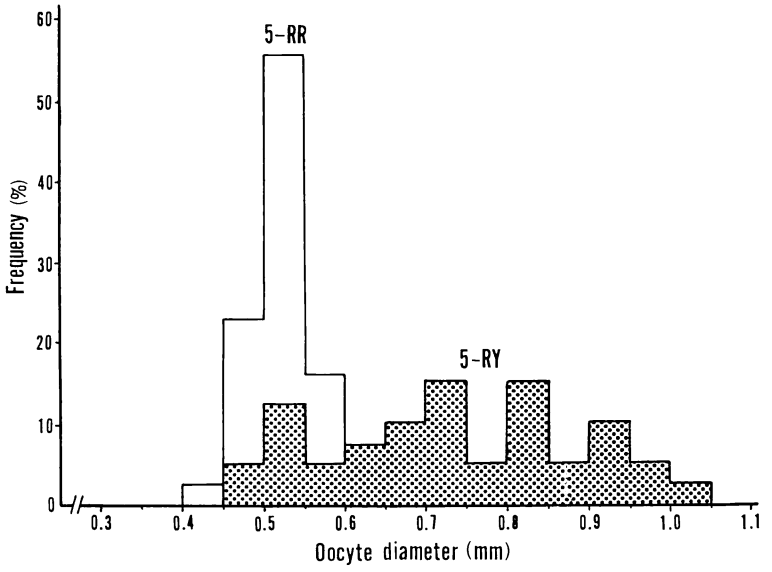


Fig. 10. Size frequencies of fresh ovarian oocytes sampled from a female (5–RR) and the hermaphroditic eel (5–RY) after 5 weekly injections of HCG followed by 2 weekly injections of salmon pituitaries.

ated by HCG and salmon pituitaries in the hermaphroditic eel was thus characterized by the occurrence of a quite asynchronous development of ovarian oocytes during the course of artificial maturation. A week after the 2nd injection of salmon pituitaries, the diameter of oocytes freshly torn from biopsied gonad fragments of the hermaphrodite (5-RY) was measured by the use of a projection photomicrometer, and its variation was compared with that of fresh oocytes in the ovarian tissue taken from a female (5-RR) receiving the same treatment (Fig. 10). By that time, whereas a majority of ovarian oocytes of the female were in the range from 450 to 550 μ in diameter, those of the hermaphrodite were much various in size, ranging from 450 to 1050 μ in diameter, without revealing any important peak in the distribution of oocyte diameters. It seems likely to occur that young oocytes reserved in the ovarian portion of the hermaphroditic gonad successively develop into vitellogenic phases and came eventually to degenerate through the stages toward full maturation, under the influence of the present artificial treatment.

Discussion

The Japanese eel, *Anguilla japonica*, is considered to be rather stable in sexual development, and no report has hitherto been concerned with hermaphroditic indications in this species of teleost so far as we know. Satoh et al. (1962) suggests that a juvenile intersexuality, which is known to occur in the European eel *Anguilla anguilla* (Kuhlmann, 1975), may not be the case for the Japanese eel. Only on rare occasions, according to Satoh et al. (1962), male eels have a few oviform germ cells, or testis-ova, in their testes. This phenomenon has also been experienced in our laboratory.

Since Japanese eels of both sexes generally possess the gonad in quite immature conditions at the commencement of their catadromous migration, the occurrence of transparent and rudimentary testicular tissue, if present, in ovaries will be hardly recognizable unless detailed histological studies are made. Such is true for the present case of hermaphroditic eel: the presence of testicular tissues in the ovarian gonad could be detectable only histologically at the time before the hormonal treatment was commenced.

Repeated treatments with gonadotropic substances resulted successfully in rapid maturation of testicular and ovarian tissues, thus making it easy to disclose an exact condition of the hermaphroditic gonad. Even after similar hormonal treatments, only 3 specimens with hermaphroditic gonads, including the one reported in this paper, could be discovered among several hundred silver females of the Japanese eel which had been studied in our laboratory during the past several years. Even with reservation mentioned above, this fact may possibly suggest that hermaphroditic conditions are of rare occurrence in female Japanese eels.

It is obvious that the treatment of the eel with exogenous hormones was not responsible for the occurrence of the intersexuality, though it was fully efficient in intensifying the condition, inasmuch as the existence of testicular tissues in the ovary had been confirmed by autopsy studies on the gonad prior to the hormonal treatment. A characteristic of the hermaphroditic gonad is that the gonad is predominantly ovarian in nature, microscopically as well as macroscopically, with discrete and compact testicular tissues localized along the distal edge of ovigerous lamellae. The tissues of both sexes were not intermingled with each other but were demarcated distinctly by connective tissue layers in all cases observed.

The condition seems to imply that the hermaphrodite is originally a female and has become provided teratologically with testicular tissues owing to undetermined factors affecting the ovary during a certain period of its sexual morphogenesis, though a spontaneous sex reversal of the gonad from either sex is not precluded. The hermaphrodite was the smallest in total length (65.9 cm) among a group of 10 females (65.9~84.5 cm, mean 74.6 cm) used in the present study. In this context it is interesting to recall the work by D'Ancona (1957) who supposed, on the basis of experimental findings on the European eel, that the sex reversal from male to female might occur spontaneously if silver eels are prevented from migrating to the sea. It has been reported also that the sex determination in the European eel may be modified by the influence of various environmental factors at the elver stage (D'Ancona, 1960; Kuhlmann, 1975). Unfortunately, information concerning the

mechanism of sex determination and differentiation of the gonad is too scanty for the Japanese eel to progress the consideration further.

The present study indicates that both the ovarian and the testicular constituents of the hermaphroditic gonad were capable of developing into maturation in response to exogenous gonadotropins. This may denote the possibility that the hermaphrodite becomes functional as to both sexes during its sexual maturation evoked by its own gonadotropic hormone(s). It is surprising, on the other hand, that the treatment with HCG could elicit considerable maturation of the ovarian as well as the testicular portions of the hermaphroditic gonad.

It has been shown that, in the European eel, HCG is effective in inducing complete maturation of the testis of young males (Boëtius and Boëtius, 1967; Lumare and Villani, 1973). However, HCG can promote but quite slightly the maturation of silver females of the European eel (Bruun et al., 1949; Villani and Lumare, 1975). In silver females of the Japanese eel used in the present study, most ovarian oocytes were about 250 μ in size and at the oil drop stage before the HCG treatment was begun, and they were barely developed to 300 μ in size of the primary yolk stage after 5 weekly injections with HCG. By the same treatment with HCG only, on the contrary, oocytes in the hermaphroditic gonad attained to 600～800 μ in size reaching the final stages of vitellogenesis. In female eels the ovarian maturity of the same degree was achieved generally after 6～8 weekly injections of salmon pituitaries subsequent to 5 injections of HCG.

The hermaphrodite was different from other females of the same experimental group in that ovarian oocytes of the former were larger in size than those of the latter and were at the primary yolk stage even before the initiation of hormonal treatment. This is interpreted as revealing an endogenous gonadotropic activity in the hermaphrodite to bring the oocytes into vitellogenesis. However, it remains to be elucidated by further studies whether the particular effect of HCG demonstrated in the hermaphrodite is elicited indirectly via the stimulation of the pituitary gland of the fish or directly on ovarian oocytes advanced in maturation.

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(Laboratory of Fresh-Water Fish-Culture, Department of Biology, Faculty of Fisheries, Hokkaido University, Minato-cho 3-1-1, Hakodate 041, Japan)

日本産ウナギの雌雄同体個体とその人為成熟

高橋　裕哉・杉本　良郎

人為成熟誘導試験のため 1976 年秋，青森県の河川で採捕した下りウナギ雌の 1 尾（全長 65.9 cm，体重 530 g）に，催熟処理開始前の開腹生検により，雌雄同体型の生殖腺を見出した．この生殖腺は外観的にも組織学的にも正常な卵巣組織を主体としたが，生殖腺の全長にわたる末端縁に無数の精巣組織の小塊を“ふさ飾り”状に有していた．この精巣組織は組織学的に正常で，結合組織層により卵巣組織から分離されていた．この個体に生殖腺刺激ホルモンを投与し，処理中定期的に生殖腺の生検をくり返した結果，雌雄同体生殖腺の卵巣部・精巣部の双方とも処理に伴う成熟の進行をみせることが確かめられたが，卵巣卵の発達が急速かつ変異に富むことも知られた．

(041 函館市港町 3-1-1　北海道大学水産学部淡水増殖学講座)