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Sex Differentiation and Development of the Gonad in the Japanese River Lamprey, *Lampetra japonica*

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Abstract

Sex differentiation and development of the gonad were examined histologically during larval, metamorphosing, and young adult stages in the Japanese river lamprey, *Lampetra japonica*, collected throughout the year in the Assabu River, southern Hokkaido.

Gonads of all ammocoetes with total lengths below 30 mm were sexually indifferent. During the subsequent course of larval growth, gonial germ cells developed to form definite cysts in all of the gonads examined, revealing the occurrence of the cystic stage in initial sex differentiation of the lamprey. In all ammocoetes with total length of 70-90 mm, germ cells in the cysts more or less showed meiotic nuclear changes, though the degree of appearance of the premeiotic cysts varied in different specimens observed. Differentiation of ovaries with growing oocytes followed the cystic stage in some larvae of total length of 70-90 mm. Testicular differentiation was observed to occur either with the breakdown of germ cell cysts and rearrangement of surviving germ cells at the cystic stage or with extensive degeneration of growing oocytes and development of gonial germ cells preexisting at the ovarian phase. It was presumed that about 10 to 20% of defined males of the lamprey had passed the latter course of testicular differentiation. A nearly 1:1 ratio of sexes seemed to be established in the lamprey exceeding 150 mm in total length. The appearance of vitellogenic oocytes and of spermatocytes was never observed even in metamorphosed, downstream migrants of the lamprey.

Since the investigation of Okkelberg¹⁾ on the brook lamprey, *Entosphenus wilderi*, several reports have been concerned with sex differentiation and development of the gonad in several species of lampreys. In these reports, much attention has been paid to the occurrence of a female or intersexual stage in early period of gonadal development: female and male germinal elements coexist together without any distinct localization in the somatic stroma of the gonad of essentially all juvenile animals. Some workers such as Okkelberg²⁾ and Champy³⁾ regarded this as a feature of sexual indetermination at an initial stage of gonadal development. However, Hardisty⁴⁾ brought forward the interpretation that it was nothing but a reflection of an innate tendency of germ cells to differentiate autonomously in the female direction in the gonad of which future sexes had already been determined.

There is paucity of information about reproductive physiology of Japanese lampreys, and detailed reports on the process of gonadal development and maturation are lacking so far as the present writers know. In the present study, the

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process of sex differentiation and development of the gonad of the parasitic Japanese river lamprey, *Lampetra japonica*, was examined histologically at various stages of the life cycle.

Material and Methods

Larval, metamorphosing, and young adult lampreys were collected in the Assabu River, southern Hokkaido, every month from April 1980 to May 1981. The specimens were fixed *in toto* in Bouin's fluid just after catching or after transporting to the laboratory. After fixation, pieces of their gonads were excised at three different levels of the body: just caudal to the liver, at the anterior end and at the posterior end of the first dorsal fin. Serial paraffin sections of gonads were cut frontally at 4 to 6 μm in thickness, and stained with Delafield's hematoxylin and eosin. Sections from the middle portion of each gonad were used to compare developmental stages of the specimens and to calculate the total number of germ cells existing in each gonad, since no essential difference was observed among the preparations from the three different places on the body.

Observations

The maximum and minimum total lengths of the specimens examined were 200 mm and 5 mm, respectively. Frequency distribution (Fig. 1) showed that

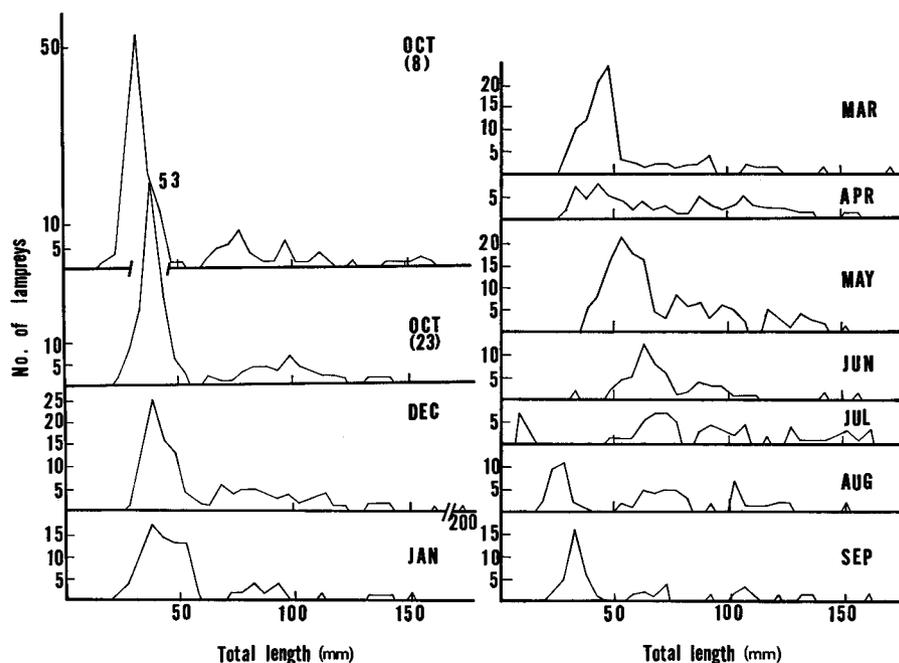
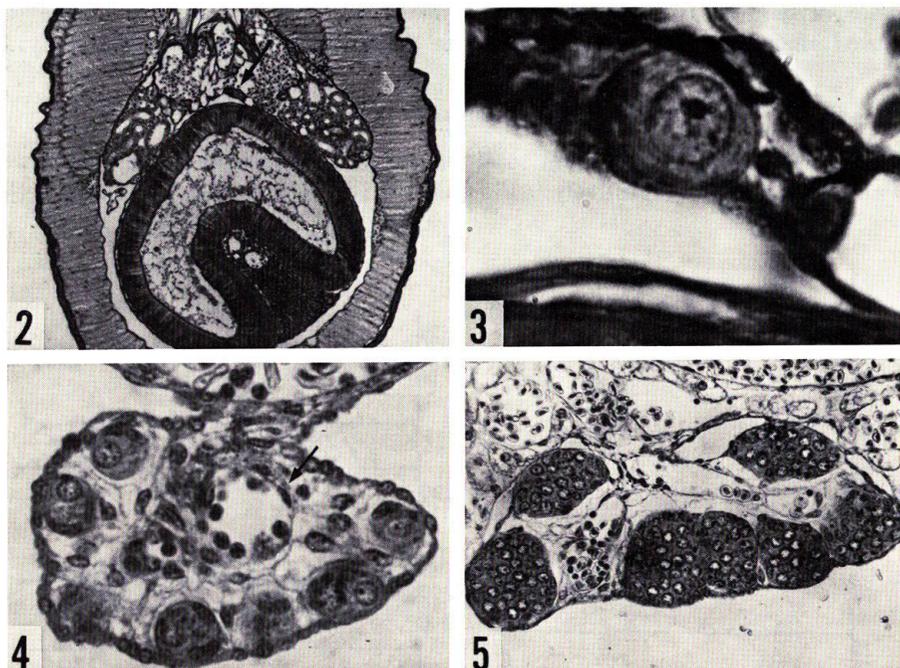


Fig. 1. Length-frequency diagrams for *Lampetra japonica* collected in the Assabu River during the period from March 1980 to January 1981.

Table 1. Percentage distribution of *Lampetra japonica* with different gonad types at different stages of body growth.

Presumed age	I	II	III	IV	
Total length (mm)	30	60	90	120	150
Stage of gonad					
Indifferent stage	100	86	11		
Cystic stage		14	75	22	
Ovary			11	69	69
Testis			3		22
Transitional stage (from ovary to testis)				9	9
No. of lampreys examined	5	14	36	36	32
					15



- Fig. 2. Transverse section of the middle region of the body of a 5-mm ammocoete. Arrow indicates position of gonad. $\times 72$.
- Fig. 3. Enlarged view of the gonad shown in the above figure, showing the presence of a single germ cell. $\times 1265$.
- Fig. 4. A luminal structure at the central region of the gonad of a 56-mm ammocoete. Arrow indicates a cavity-like structure. $\times 560$.
- Fig. 5. A typical cystic gonad of a 73-mm ammocoete. No germ cells are in the meiotic prophase. $\times 185$.

each of the groups of 0~50 mm, 50~95 mm, 95~125 mm, 125~155 mm and 155~200 mm in total length was presumed to correspond to one year class, as was relatively clear from the samples of July and August. Metamorphosing specimens were found to be around 150 mm in total length. The duration of the ammocoete period was thus estimated to be 4 to 5 years. The appearance in July of a new year class with a mode of the total length at 7.5 mm suggested that, in the locality selected in the present study, the spawning period of *Lampetra japonica* was around June. Examination of the maturity of upstream migrants of the lamprey confirmed this suggestion. Furthermore, it was suggested that the growth of ammocoetes was rapid during spring and summer months and almost stagnant in autumn and winter.

The rate of appearance of lampreys with gonads at different developmental stages is shown in Table 1 as a function of the total length of the animal. Gonads of all ammocoetes below 30 mm in total length were in the sexually indifferent stage. Primordial gonads, in which only a few germ cells existed, were first detected in larvae of 5 mm long as a single median swelling of the coelomic epithelium (Figs. 2, 3). The germ cells were large in size, about 15 μ m in diameter, with round nuclei, and their cytoplasm was lightly stained. Even at that earliest stage of development, bilateral symmetrical arrangement of the gonad, which was common in teleost fishes, was not observed. In gonads of many larvae of about 40 mm in total length, presumably half a year after hatching, some cysts of gonial

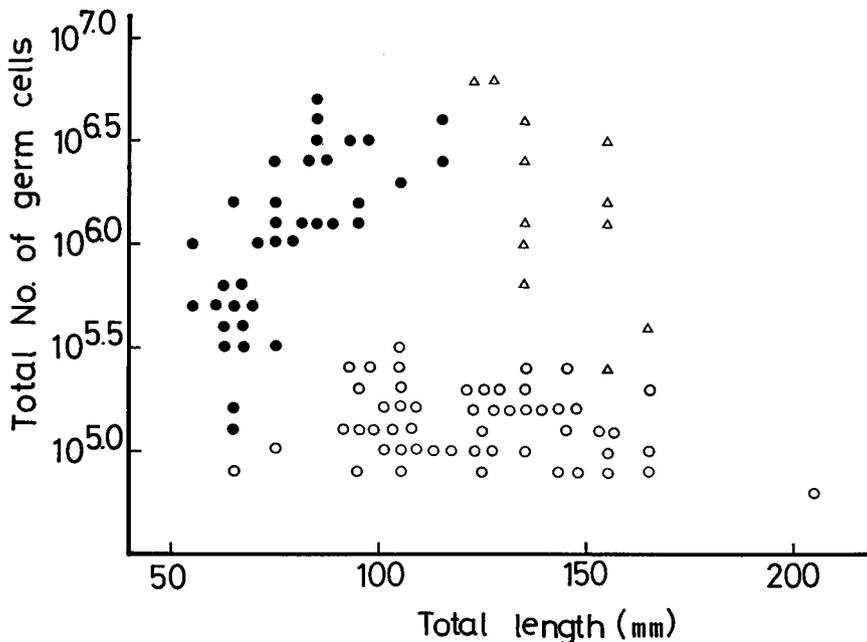


Fig. 6. Relationship between the total length of fish and the total number of germ cells of three different types of gonads in *Lampetra japonica*. o, ● and Δ indicate ovary, cystic gonad and testis, respectively.

germ cells as well as solitary ones appeared (Fig. 4), but these cysts were small in size and mitotic division of germ cells was scarcely observed. On some occasions, a cavity-like structure was seen to occur in the central region of the gonad.

Out of 14 ammocoetes examined at total length of 30 to 60 mm, 12 (86%) had gonads at the indifferent stage, while the remaining 2 (14%) had gonads in which definitely organized cysts of gonial germ cells were prominent while solitary gonia occurred only occasionally (Fig. 5). In the present study, gonads of the latter type were tentatively designated as being in the "cystic" stage. The larvae measuring 60~90 mm in total length were observed invariably to have gonads at the cystic stage, with a number of large cysts possibly resulting from rapid division of gonial germ cells. It was estimated by calculation that, at the cystic stage of development, about 5,000 to 20,000 cysts of germ cells were present in each gonad, amounting to about 1.2×10^5 to 15.8×10^5 germ cells in

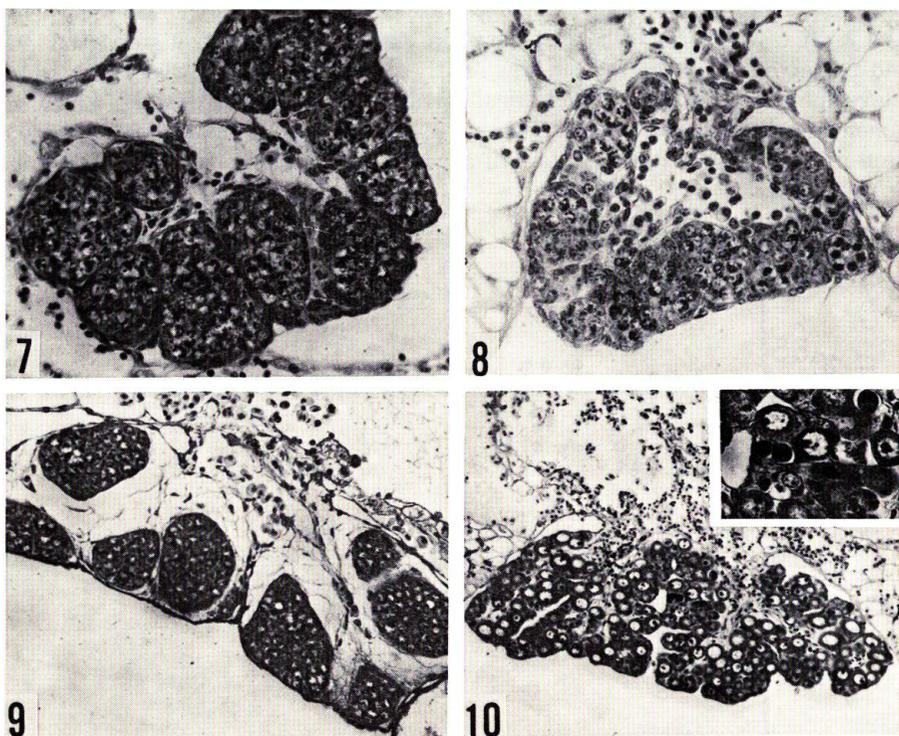


Fig. 7. A cystic gonad of a 69-mm ammocoete. Almost all germ cells in every cyst are in the meiotic prophase. $\times 235$.

Fig. 8. A cystic gonad of a 53-mm ammocoete. Germ cells in some cysts are in the meiotic prophase and those in others are still in the gonial stage. $\times 300$.

Fig. 9. A cystic gonad of a 112-mm ammocoete. Each cyst contains both premeiotic germ cells and gonial ones. $\times 190$.

Fig. 10. Ovary just after differentiation of a 96-mm ammocoete. $\times 105$.

Inset: Atretic oocytes occurring in the ovary. $\times 260$.

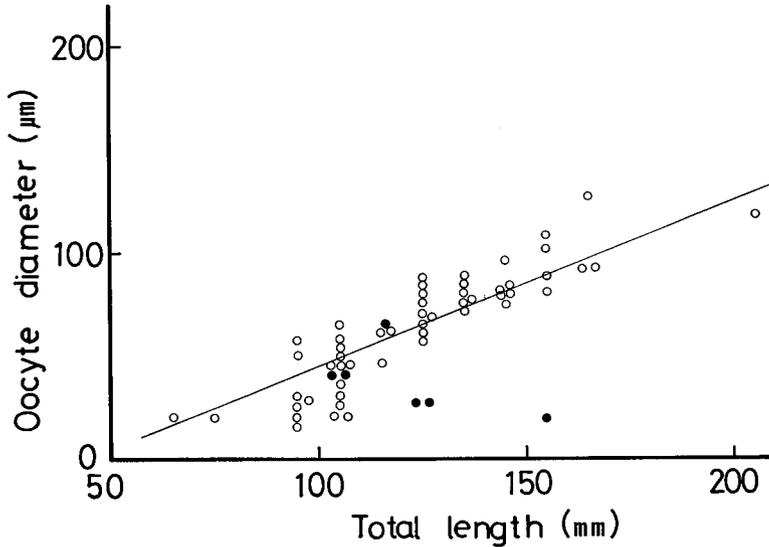


Fig. 11. Relationship between the total length of fish and the oocyte diameter of female lamprey. ○ and ● indicate an individual fish having ovary and transitional gonad with degenerating oocytes, respectively.

total, and that 8 to 512 gonial germ cells were contained in each cyst in which their mitoses eventually occurred at least 9 times (Fig. 6). Several patterns of the onset of meiosis in the cyst were observed in the gonad of these larvae. In gonads of 7 ammocoetes, germ cells in almost all the cysts had synchronously entered into the meiotic prophase (Fig. 7), while in those of another 3 larvae cysts of gonial germ cells existed in larger numbers than those of premeiotic ones (Fig. 8). In these two cases, germ cells in a given cyst were seen to have entered into the meiotic prophase synchronously. On the other hand, in gonads of the remaining 4 ammocoetes, the onset of meiosis in the cyst was not synchronous, germ cells in the premeiotic and gonial stages both occurring at various ratios in the same cyst (Fig. 9). In all these cases, only a few solitary gonia were present in the gonads of a few specimens.

Out of 36 ammocoetes examined at length of 60~90 mm, 4 specimens had gonads of definite ovarian nature which were filled with auxocytes of similar sizes together with only a few cysts of premeiotic germ cells (Fig. 10). Out of 36 ammocoetes examined at length of 90~120 mm, 8 still had gonads containing germ cell cysts only, whereas 25 possessed ovaries with growing oocytes and were regarded as definite females. At that period of body growth, the total number of germ cells in gonads in the cystic stage reached about 39.8×10^5 , but that in the ovary was evidently fewer than that in the cystic stage (Fig. 6). Growing oocytes in the ovary tended to decrease in number gradually during the course of ovarian development until after metamorphosis. During the successive growth periods through metamorphosis to the young adult stage, ovarian oocytes continued to

grow larger with increasing stainability by basophilic dyes in their cytoplasm, but they never entered into the period of vitellogenesis even in the metamorphosed, downstream migrants of the lampreys. There was, however, a conspicuous correlation between oocyte diameter and body length during these periods (Fig. 11). The correlation is represented by a regression line, $Y=8.2X-38$ ($r=0.78$, $p<0.01$; Y: oocyte diameter, μm ; X: total length, cm), by the method of least squares.

In the lamprey examined in this study, differentiation of the testis generally occurred after some delay as compared with that of the ovary, and was evident in 7 (22%) out of 32 specimens observed at length of 120 to 150 mm, though only one among 36 specimens measuring 60~90 mm in total length was shown to have the gonad differentiating in the male direction (Tab. 1). The most distinctive histological characteristics seen in the differentiating testes were relatively few germ cells and many somatic cells as compared with those at the cystic stage, and a complex infolding of the surface of gonad along which gonial germ cells were distributed singly or in small clusters (Fig. 12).

Two patterns in the process of testicular differentiation were discernible in these specimens. Testicular differentiation by the first pattern started in gonads

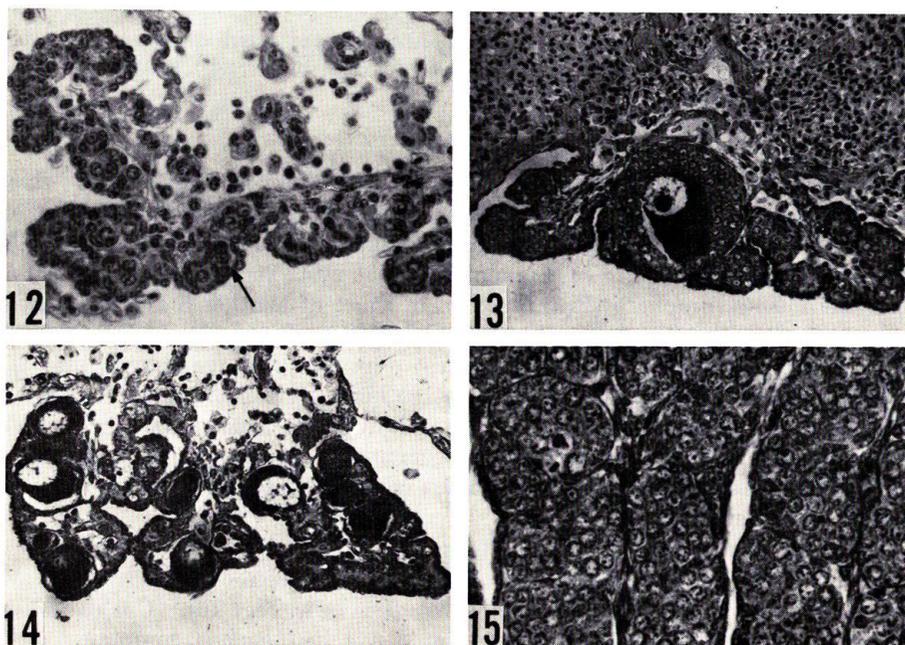


Fig. 12. Testis of a 132-mm ammocoete, in an initial phase of differentiation. The somatic elements are compactly arranged along the peripheral region of the gonad. Arrow indicates germ cell. $\times 330$.

Fig. 13. Testis of a 151-mm ammocoete, showing the presence of an oocyte in the center of a cyst. $\times 190$.

Fig. 14. Transitional gonad of a 105-mm ammocoete, indicating an advanced lobation of gonad and the existence of many oocytes in the same gonad. $\times 210$.

Fig. 15. Testis of a 144-mm downstream migrant after metamorphosis. $\times 340$.

of the cystic stage. In these gonads, cysts of gonial or premeiotic germ cells became broken up by the invasion of surrounding somatic cells, eventually causing the constituent gonial germ cells to be intermingled with the somatic cells (Fig. 12). In the differentiating testes, germ cells became considerably fewer in number as compared with those in the cystic gonads, possibly by their degeneration occurring through the course of testicular differentiation. In testes which had differentiated through such a course, gonial germ cells frequently showed an active multiplication to form spermatogonial cysts arrayed along the peripheral region. On many occasions, however, a few premeiotic or oviform germ cells were observed to exist in a few spermatogonial cysts (Fig. 13).

Testicular differentiation by the second pattern was observed to be initiated in typical ovaries with many developing oocytes. The gonads were characterized, in the course of testicular differentiation, by having many oocytes undergoing degeneration and some gonial germ cells arranged along the complicatedly infolded surface (Fig. 14). It was remarked that the oocytes existing in these gonads were generally smaller in size than those in normal ovaries (Fig. 11). Differentiating testes of this type were encountered in 3 (9%), 3(9%) and 1(7%) ammocoetes at lengths of 90~120 mm, 120~150 mm, 150~200 mm, respectively (Tab. 1). It was estimated that about 10 to 20% of the defined male lampreys had testes developing through this pattern of differentiation. Sex ratio became nearly 1:1 in the lampreys exceeding 150 mm in total length when testicular differentiation of the two patterns had been completed in all the ammocoetes that were regarded as males. Later on, spermatogonia underwent slow multiplication in testicular cysts. In downstream migrants after metamorphosis, spermatogonial divisions were relatively frequent in their testes, which were packed with numerous spermatogonial cysts of uniform size (Fig. 15).

Discussion

On the basis of the observations described in the present study, the patterns of sex differentiation and development of the gonad in the lamprey, *Lampetra japonica*, are schematically illustrated in Fig. 16. All of the gonads of the ammocoetes below 55 mm in total length (1 year class) were sexually indifferent. They subsequently developed into the cystic stage which characterized an initial phase of gonadal sex differentiation in the lamprey studied. Differentiation of the ovary, which was marked by synchronous transition of germ cells into meiotic prophase and by successive development into auxocytes, was initiated in a portion of larvae measuring 70 to 90 mm in total length (2 year class) and terminated in most larvae measuring about 100 mm in total length (3 year class). No oocytes in the process of vitellogenesis were observed even in ovaries of the metamorphosed, downstream migrants.

The most conspicuous feature of gonadal sex differentiation in the lamprey was that two different types of testes were discernible in the process of their differentiation (Fig. 16). Testicular differentiation occurred either in gonads of the cystic stage by rearrangement of germinal and somatic elements, or in those differentiated as definite ovaries through a transitional stage. In the latter type the duration of the ovarian phase was different to some extent among future

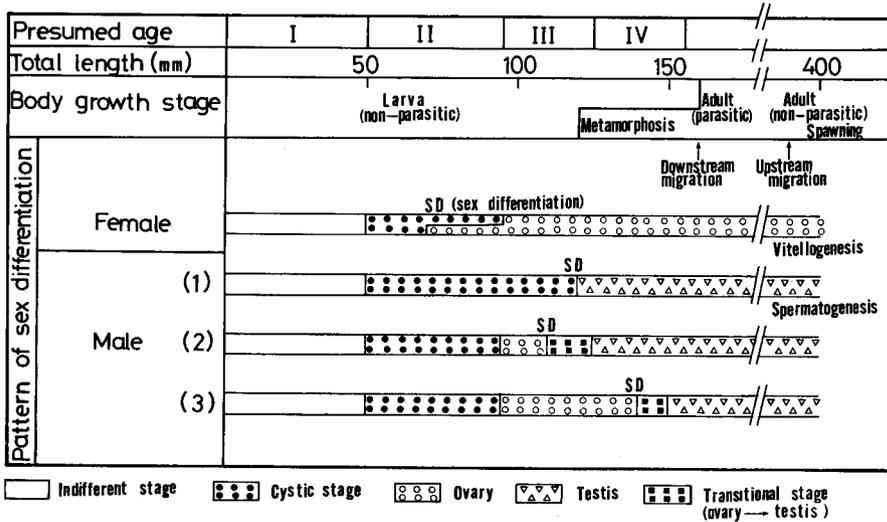


Fig. 16. Schematic illustration of different patterns of gonadal sex differentiation in *Lampetra japonica*.

males: it was longer in some of them, lasting until up to about 140 mm in total length. It was estimated in this study that testes of about 10 to 20% of males had differentiated through transition from defined ovaries. Hardisty⁵⁾ also described that, in *Lampetra planeri*, about 10% of all male gonads were derived from the gonads where oocytes were dominantly developing.

It has been reported that a female or intersexual stage occurs in the course of testicular differentiation in some species of lampreys such as *Lampetra fluviatilis*⁶⁾ and *L. lamotteni*²⁾. Some interpretations have been given to the appearance of transitional gonads in lampreys. Okkelberg¹⁾, who observed both gonial germ cell cysts and oocytes coexisting in the gonad of *Entosphenus wilderi* during the process of sex differentiation, described the state of the gonad as the stage of sexual indetermination. On the other hand, Hardisty⁵⁾ regarded the appearance of oocytes in male gonad of *Lampetra planeri* as a reflection of an innate tendency of bipotential germ cells to develop autonomously in the female direction, and thought that testicular differentiation had already terminated in these gonads at that stage. It is interesting to note that, in the lamprey of the present study, the oocytes seen in gonads at an initial phase of transition into testicular structure remained generally smaller in size than those in typical ovaries of the ammocoetes of a comparable size, notwithstanding the time of occurrence of the transition. This fact seems to indicate that the intersexual gonads have been determined to develop as testes at quite an early period of the ovarian phase. Furthermore, the occasional appearance of premeiotic germ cell cysts or developing oocytes in the differentiated testes observed in the present study seems to point to an innate female potential of germ cells of the lamprey, which may support the aforementioned view proposed by Hardisty⁵⁾.

The testis just after differentiation was characterized by having a small number

of germ cells and a large number of somatic cells in comparison with the cystic gonad. This may mean that a large number of germ cells had been lost during the course of testicular differentiation. Earlier authors¹⁾⁷⁾ considered that gonial germ cell cysts in the cystic gonad persisted intact to become spermatogonial ones in testicular gonads, and that the gonad with germ cell cysts developing beyond a certain size was regarded as the future testis. However, this was not the case for the Japanese lamprey in the present study. In testicular gonads newly differentiated from cystic gonads, gonial germ cells were seen to exist singly or in clusters of several cells interspersed among somatic elements, which denoted the breakdown of preexisting germ cell cysts and the rearrangement of the surviving gonial germ cells. Later on, the spermatogonia began to multiply to form new spermatogonial cysts in defined testes.

The present study failed to decide future sexes of ammocoete larvae with gonads of the cystic stage, though three types were distinguishable histologically among these gonads in terms of the degree of the appearance of premeiotic germ cell cysts. It was observed, however, that in some ammocoetes with total lengths over 100 mm, when differentiation of ovaries had been completed in almost all the future females, gonads remained at the cystic stage, which contained gonial germ cell cysts as predominant constituents. Accordingly, it is highly likely that the cystic gonads of this type may have destined to differentiate into testes in a latter period of body growth.

Another important problem concerning morphogenesis of the testis in lampreys is the origin and significance of somatic cells occurring in relatively large amounts in differentiating testes. In this context, Hardisty⁴⁾ suggests that an increment in amount of somatic cells in the testes may result from (1) a concentration effected by a decrease in size of male gonads, (2) recruitment by proliferation of cells of the peritoneal epithelium, and (3) the survival of somatic elements of germ cell follicles after complete degeneration of germ cells. It seems possible also in the present study that somatic elements at least in the central region of the differentiating testes came to contain follicular somatic cells that survived after the degeneration of germ cells and those in the peripheral region derived mainly from the peritoneal epithelium. Hardisty⁵⁾ assumed the somatic elements to play a certain role in testicular differentiation, though he stated later that the increment of somatic elements was a result rather than a cause of frequent degeneration of germ cells during testicular differentiation⁴⁾. It has repeatedly been shown that, in teleost fishes, somatic cells of the gonad may have an important role in the determination of gonadal sexes⁸⁾. The problem remains to be solved by further studies, including experimental ones.

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