Bull. Fac. Fish. Hokkaido Univ.
34(4), 279–290. 1983.

Sex Differentiation and Development of the Gonad in the Sand Lamprey, *Lampetra reissneri*

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Abstract

The process of sex differentiation and development of the gonad was examined histologically in the sand lamprey, *Lampetra reissneri*, during its larval, metamorphosing, and adult periods.

Gonads of ammocoetes less than 30 mm in total length were invariably in the sexually indifferent stage. Ammocoetes having sexually indifferent gonads appeared during successive periods of body growth up to the 100–130 mm stage. Gonads with varying numbers of developing oocytes together with degenerating ones came to appear in some animals at the 30–50 mm stage. Some gonads of the ovarian type developed subsequently into typical ovaries which had completed their differentiation at the 60–70 mm period, suggesting the establishment of the female sex at that period. It was remarked that the differentiation of ovaries occurred directly from sexually indifferent gonads in a majority of females, without passing through the cystic stage which characterized the initial phase of gonadal sex differentiation in the Japanese river lamprey, *Lampetra japonica* (Fukayama and Takahashi, 1982). Testicular differentiation was observed to occur either directly from indifferent gonads, eliminating the cystic stage in most cases, or indirectly from ovaries in which extensive degeneration of growing oocytes was prominent. It was presumed that about 10 to 20% of defined males of the lamprey were derived through sex reversal from animals which had possessed typical ovaries. The time of occurrence of testicular differentiation was quite different in different individuals, ranging from the 50–60 mm to the 140 mm stage of body growth. A nearly 1:1 ratio of sexes seemed to be established by the 160–190 mm stage of the larval period. The results of observations on nonparasitic sand lamprey, *Lampetra reissneri*, were compared with those on parasitic Japanese river lamprey, *Lampetra japonica*, in terms of the lability of sexuality during the larval period.

Conspicuous growth of oocytes with advancing vitellogenesis and accelerated divisions of spermatogonia were observed to start in female and male lamperys, respectively, during metamorphosis. These findings suggest a possible interrelation between metamorphosis and sexual maturation of gonads in the sand lamprey.

It is well known that lampreys include both parasitic and nonparasitic species (cf. Hardisty, 1979). The term "paired" (Zanandrea, 1959; Hardisty and Potter, 1971c) or "satellite" (Vladykov and Kott, 1979) species has been applied to a combination of parasitic and nonparasitic species which are almost identical morphologically and are believed to have derived from the same ancestral parasitic species. To date, several reports have been concerned with sex differentiation and development of the gonad in various species of lampreys (Okkelberg, 1914, 1921; Champy, 1924; Busson-Mabillot, 1967; Hardisty, 1965, 1969, 1970, 1971, 1979;

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Lewis and McMillan, 1965; Fukayama and Takahashi, 1982; Seagle and Nagel, 1982; Beamish and Thomas, 1983). In some of these reports, considerable interest has been taken in the development of gonads in relation to the life cycle of some "paired" species, showing that accelerated gonadal development initiates during metamorphosis in nonparasitic species whereas it begins much later in parasitic ones (Hardisty, 1965, 1970, 1971; Beamish and Thomas, 1983).

The sand lamprey, *Lampetra reissneri*, is a nonparasitic species which forms the "paired" species with the parasitic Japanese river lamprey, *Lampetra japonica*. Some characteristic aspects of the sex differentiation and development of the gonad in the Japanese river lamprey have been described in an earlier paper (Fukyama and Takahashi, 1982). In the present study, the process of sex differentiation and development of the gonad in the nonparasitic sand lamprey, *Lampetra reissneri*, was examined histologically at various stages of the life cycle.

Material and Methods

Larval, metamorphosing, and adult specimens of the sand lamprey, *Lampetra reissneri*, were collected in the Ohno River, southern Hokkaido, at least once a month during a period from April 1980 to June 1981. The specimens were fixed *in toto* in Bouin's fluid soon after the capture or after transporting them to the laboratory. The fixed bodies were crosscut at levels just caudal to the liver, and just anterior and posterior to the first dorsal fin, which contained the anterior, middle and posterior portions of the gonads, respectively. Serial paraffin sections of these parts of the body were cut frontally at 4 to 6 μm in thickness, and stained with Delafield's haematoxylin and eosin. Since no essential differences were observed in gonad histology among the three different portions of the body, sections from the middle portion of each gonad were selected to compare developmental stages of the specimens and to calculate the total number of germ cells existing in each gonad. Stages of metamorphosis were determined according to the criteria proposed by Youson and Potter (1979).

Observations

Frequency distribution of the total lengths of all lampreys collected did not suggest the demarcation of a class for each year of the animal or the duration of the

Table 1. Percentage distribution of different gonad types in *Lampetra reissneri* at different stages of their body growth.

Total length (mm)	30	50	60	70	100	130	160	190
Stage of gonad								
Indifferent stage	100	50	47	35	11	13		
Cystic stage		18	19	6	9	13	14	
Ovary		32	26	53	42	44	45	53	100
Testis			5	6	29	17	41	42	
Transitional stage (from ovary to testis)			3		9	13		5	
No. of lampreys examined	2	22	38	17	82	30	29	19	6

larval period. Five different stages of gonads were histologically distinguishable among these lampreys of different total lengths (Table 1).

Gonads of ammocoetes smaller than 30 mm in total length exclusively comprised a few solitary or clustered gonial germ cells arranged peripherally around a sparse stromal tissue (Fig. 1), and were regarded as being at the sexually indifferent stage of gonadogenesis. As in the case of Japanese river lamprey, *Lampetra japonica*,

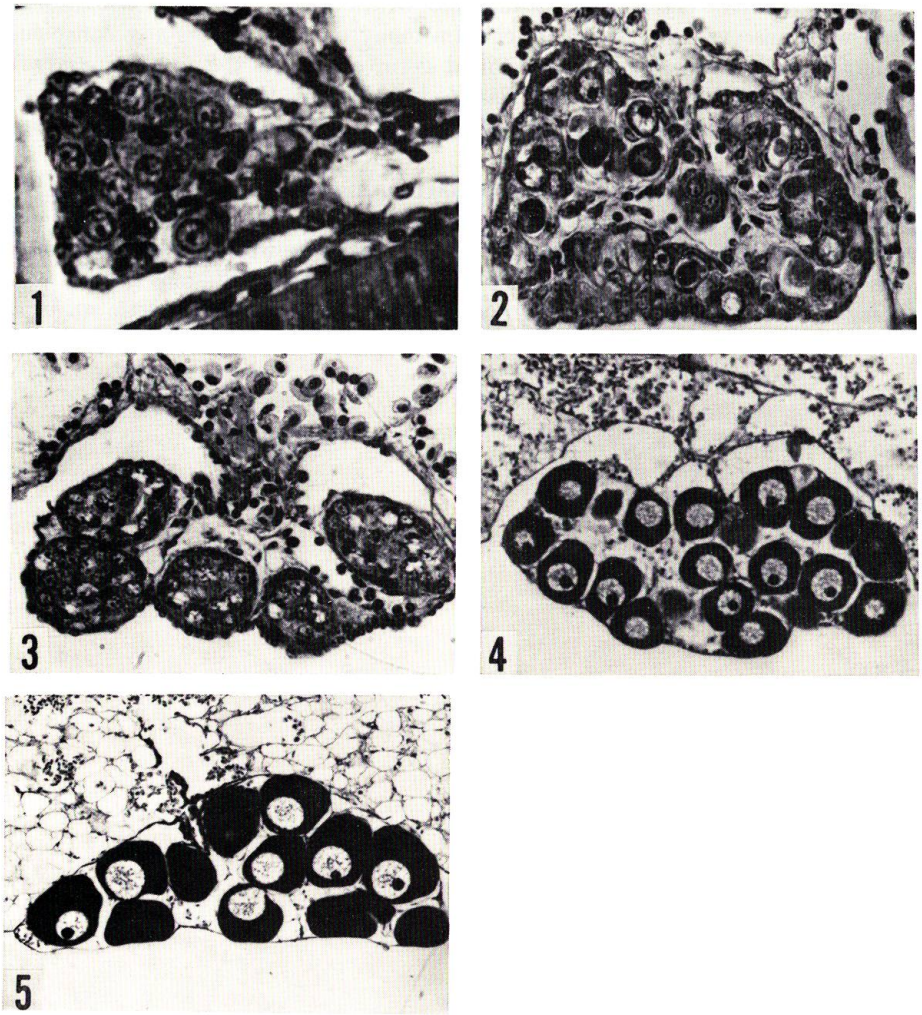


Fig. 1. Sexually indifferent gonad of a 30 mm ammocoete. ×710.
Fig. 2. Gonad of the ovarian type of a 40 mm ammocoete, containing many oocytes undergoing conspicuous degeneration. ×400.
Fig. 3. Typical cystic gonad of a 52 mm ammocoete, with germ cells in the meiotic prophase in each cyst. ×390.
Fig. 4. Typical ovary of a 74 mm ammocoete. ×125.
Fig. 5. Developing ovary of a 134 mm ammocoete. ×83.

bilateral arrangement of gonads was not observed in the sand lamprey, *Lampetra reissneri*, even at this earliest stage of gonadal development. Gonads of the sexually indifferent type were observed to occur in larger lampreys at least up to the 100–130 mm stage (Table 1). Out of 22 larvae of the 30–50 mm period of body growth, 11 had gonads of the sexually indifferent stage, while 7 had gonads of the ovarian type in which varying numbers of small oocytes measuring about 20 μm in diameter were developed. At the same time, however, oocytes undergoing atretic changes were also observed very frequently in these ovaries (Fig. 2). In extreme cases, gonads were observed to exclusively contain many oocytes undergoing conspicuous degeneration together with healthy gonial germ cells. In the present study the gonads of the latter type were also classified as ovaries, though it was difficult to assume that all of these gonads were destined to develop eventually into definite ovaries.

Four out of 22 lampreys of the 30–50 mm stage had gonads in which well-organized cysts of gonial germ cells were seen to be arranged around a sparse stromal tissue (Fig. 3). In general, the germ cell cysts were almost equal in size in the same gonad, but they were quite different in size in different gonads of the lampreys in the same stage of body growth. Gonads typical of the cystic stage were observed to occur in every stage of total length up to at least the 130–160 mm stage,

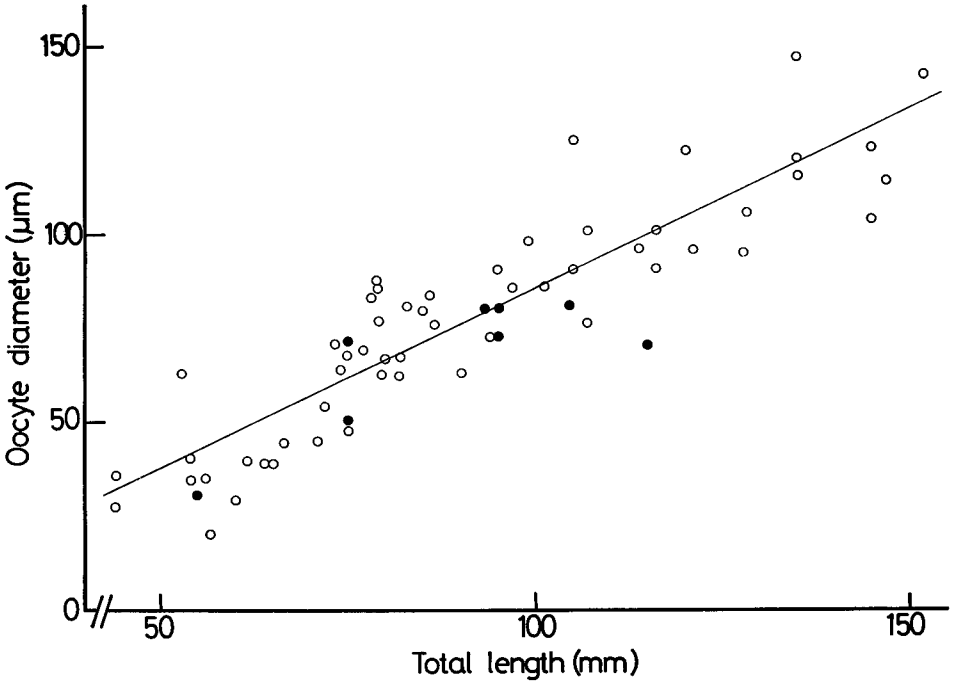


Fig. 6. Relationship between the total length of fish and mean oocyte diameter in ovaries of larval females. Marks in the figure indicate idividual fish having ovary (○) and transitional gonad with degenerating oocytes (●).

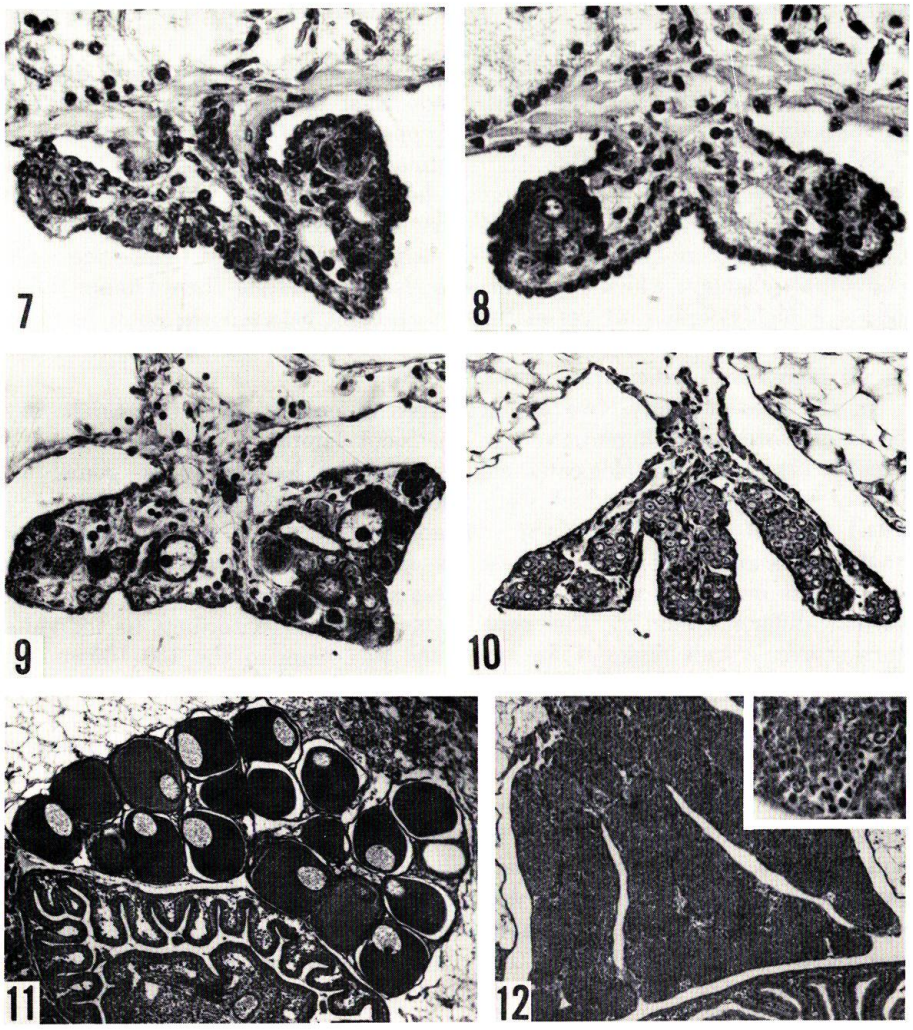
though the rate of appearance of the gonads was always relatively low (6–19%) in all the stages observed (Table 1).

Typical ovaries, which were characterized by containing many developing oocytes and a few degenerating ones, made their appearances at the 60–70 mm stage of body growth (Fig. 4). The rate of appearance of the ovaries reached a level of 53% at that stage (Table 1). Through the successive stages of growth, ovarian oocytes gradually grew larger, showing increasing stainability to basic dyes in their cytoplasm (Fig. 5). It was remarked, however, that they never entered into the period of vitellogenesis before the lampreys came to exhibit metamorphic changes, which began in some lampreys larger than 140 mm in total length. A highly significant correlation was present between the total length and oocyte diameter in larval lampreys smaller than about 140 mm in total length (Fig. 6; regression coefficient $r=0.91$, $p<0.001$).

In the present study, two distinct types of gonads were discernible in the process of testicular differentiation in the sand lamprey. Gonads of the first testicular type were histologically characterized by having a few gonial germ cells and somatic elements which were compactly arranged along the periphery of lobated gonadal tissue (Figs. 7, 8). Although only a few degenerating oocytes appeared occasionally in some of these gonads, the gonads were assumed to have been derived directly from those of the sexually indifferent stage. The time of testicular differentiation of this type seemed to vary according to individuals, occurring over a wide range of sizes from the 50–60 mm to the 130–160 mm stage (Table 1, testis).

Gonads of the second testicular type were observed to originate from those of the typical ovarian type. They showed a compact arrangement of somatic cells along the periphery of the lobated tissue, where resting gonial germ cells were also distributed sporadically (Fig. 9). Gonads of the second testicular type were easily distinguished from those of the first type in that many degenerating oocytes were found mounted among sparse stromal cells of the former (Fig. 9). In addition, many of these degenerating oocytes were almost equal in size to healthy oocytes seen in typical ovaries (Fig. 6). The gonads were thus considered to be in the process of transition from ovaries to testes. They were encountered in ammocoetes in the 50–60 mm, 70–100 m, 100–130 mm and 160–190 mm stages at ratios of 3, 9, 13 and 5%, respectively (Table 1, transitonal stage). It was estimated that about 10 to 20% of the eventually defined males of the sand lamprey had testes which had passed through this pattern of testicular differentiation. During the larval periods, testes showed quite gradual development to form many spermatogonial cysts (Fig. 10), though multiplication of spermatogonia progressed quite slowly up to the latter half of the metamorphic period. In metamorphosing or metamorphosed lampreys, structural differentiation of the testis was seen to have been completed, any sign of sexual transition being no longer present in the testes of these animals.

Lampreys in the process of metamorphosis, ranging from 140 to 200 mm in total length, could be collected only during a period from mid-June to late September. Even in female lampreys which were larger than 170 mm in total length and did not show any sign of metamorphosis, oocytes remained less than about 140 μm in diameter. On the contrary, in female lampreys during metamorphosis, oocytes showed an initiation and rapid progress of vitellogenesis accompanied



Figs. 7 and 8. Differentiating testes of a 75 mm ammocoete (Fig. 7) and a 104 mm ammocoete (Fig. 8). Somatic elements are compactly arranged along the peripheral region of the gonad. Fig. 7, ×480. Fig. 8, ×580.

Fig. 9. Transitional gonad of a 59 mm ammocoete, revealing a lobated structure typical of the testis. ×340.

Fig. 10. Developing testis of a 167 mm ammocoete, showing the formation of many small cysts of germ cells. ×200.

Fig. 11. Ovary of the lamprey at the metamorphoisis stage 6. ×48.

Fig. 12. Testis of the lamprey at the young adult stage. ×65. Inset: spermatogonia undergoing active multiplication. ×225.

by a remarkable increase in diameter (Fig. 11). The growth of oocytes during the metamorphic period progressed independently of the total length of lampreys, and was prominent particularly in the latter stages of metamorphosis (Fig. 13). On

the other hand, oocytes decreased in number gradually by degeneration through the larval and metamrophic periods, amounting to about 3000 at metamorphic stage 7 and at the young adult stage, which closely corresponded to the total number of oocytes found in lampreys during the spawning period.

In the testes of metamorphosing male lampreys, spermatogonia underwent active multiplication irrespective of either the total length of the animals or the time of occurrence of testicular differentiation, resulting in an increase in number and size of the spermatogonial cysts which filled up the testes (Fig. 12). However, in the testes of ammocoetes, even when the animals reached over 160 mm in total length, spermatogonia still remained quiescent. The accelerated development of the testes was prominent particularly in the latter stages of metamorphosis as in the case of oocyte growth in metamorphosing female lampreys. It was estimated by calculation that, at the young adult stage, each testicular cyst eventu-

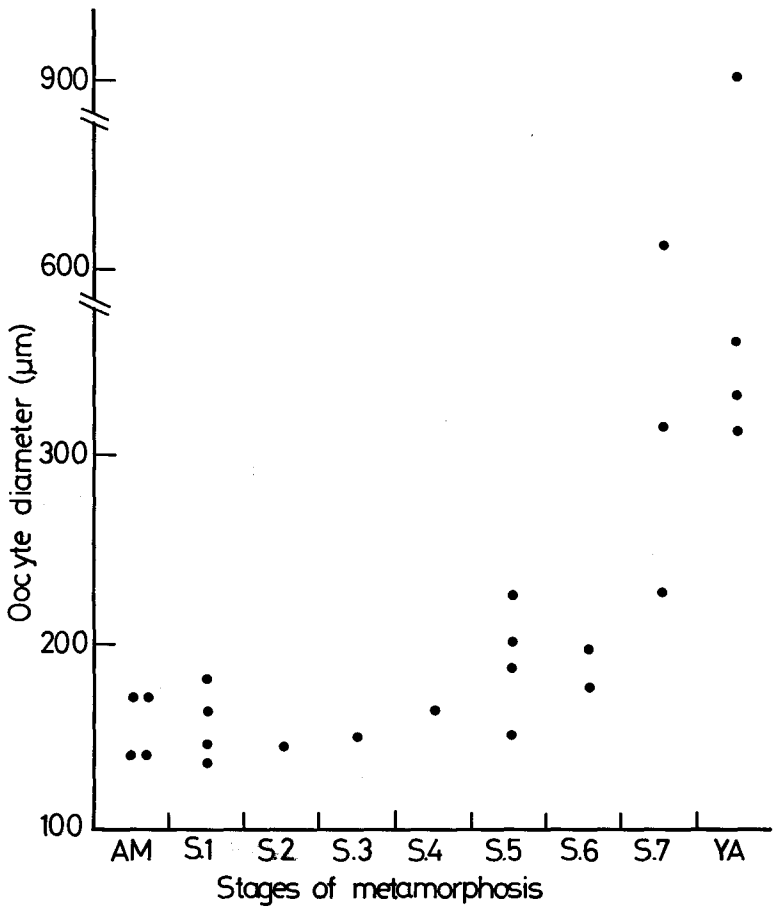


Fig. 13. Relationship between mean oocyte diameter and metmorphosis stages (S.1–S.7) in female *Lampetra reissneri*. AM, ammocoete stage; YA, young adult stage.

ally came to comprise about 8000 gonial germ cells which were produced through at least 9 synchronous mitoses.

Discussion

On the basis of the observations made in the present study, patterns of sex differentiation and development of the gonad in the sand lamprey, *Lampetra reissneri*, are schematically illustrated in Fig. 14. All of the gonads of ammocoetes less than 30 mm in total length were sexually indifferent.

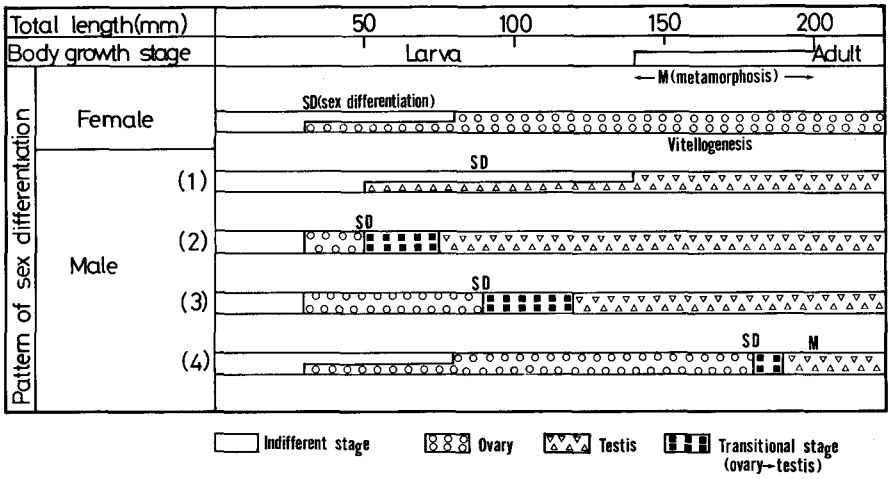


Fig. 14. Schematic illustration of different patterns of gonadal sex differentiation in *Lampetra reissneri*.

In the Japanese river lamprey, *Lampetra japonica*, which is regarded as the "paired" parasitic form of the sand lamprey, gonial germ cells multiply actively to form prominent cysts in sexually indifferent gonads, and thus the cystic stage characteristically follows the sexually indifferent stage of gonadal development (Fukayama and Takahashi, 1982). In the sand lamprey, by contrast, cysts of gonial germ cells appear only rarely in sexually indifferent gonads, and instead some oocytes occur frequently in many of these gonads, indicating that the differentiation of gonads of the ovarian type follows directly from the indifferent stage without passing through the cystic stage. Subsequently, in some individuals, ovarian oocytes continue their gradual growth during the larval period and show a rapid growth accompanied by vitellogenesis during metamorphosis, thus establishing the female sex in these lampreys.

As in the case of the Japanese river lamprey (Fukayama and Takahashi, 1982), two types of testes were discernible in the process of differentiation in the sand lamprey, indicating that testicular differentiation occurs either directly from sexually indifferent gonads or indirectly from typical ovaries through a transitional stage. The time of occurrence of testicular differentiation varies according to

individuals in both cases. It was estimated in the present study that testes of about 10 to 20% of the eventual males differentiated through a transition from ovaries. For other lampreys such as *Lampetra planeri* (Hardisty, 1965) and *Lampetra japonica* (Fukayama and Takahashi, 1982), similar estimates have been given for the occurrence of males which develop through a female phase.

The appearance of oocytes in the gonad during testicular differentiation has been reported in various species of lampreys such as *Entosphenus wilderi* (Okkelberg, 1921), *Petromyzon marinus* (Hardisty, 1965, 1971), *Lampetra planeri* (Hardisty, 1965, 1970, 1971), *Lampetra fluviatilis* (Hardisty, 1970, 1971), *Lampetra lamottenii* (Okkelberg, 1914), *Lampetra japonica* (Fukayama and Takahashi, 1982), and *Ichthyomyzon gagei* (Beamish and Thomas, 1983). Okkelberg (1921) considered the gonads with occasional oocytes to be in a stage of sexual indetermination in *Entosphenus wilderi*, while Hardisty (1965) thought that some innate potential to develop in the female direction persisted in some germ cells of differentiated testes in *Lampetra planeri*. It is interesting to note that, in the sand lamprey, oocytes seen in gonads at an initial phase of transition from ovaries to testes were comparable in size to those of the typical ovaries of ammocoetes of a corresponding size. This contrasts sharply with the case of the Japanese river lamprey, in which oocytes appearing in transitional gonads were generally smaller than those seen in typical ovaries of ammocoetes of a corresponding size (Fukayama and Takahashi, 1982). These facts seem to indicate that, at least in some of the eventual males of the sand lamprey, gonads of ammocoetes may assume a definite female phase before they differentiate into defined testes through a sexual reversal characterized by an extensive degeneration of oocytes. A noticeable tendency of germ cells to develop in the female direction in the present species of lampreys is further reflected in the fact that premeiotic and developing oocytes occurred more or less in almost all the gonads at an early phase of their differentiation from sexually indifferent gonads to testes.

It has generally been accepted that living nonparasitic species of lampreys are derived from ancestral parasitic species that have a close systematic relationship to the living parasitic species (Hardisty and Potter, 1971c; Hubbs and Potter, 1971; Vladykov and Kott, 1979). Along this line of consideration, Vladykov and Kott (1979) claimed that the same evolutional relationship might exist vetween the Japanese river lamprey, *Lampetra japonica*, and the sand lamprey, *Lampetra reissneri*, and proposed the term "satellite" species to describe such a relationship. The features of gonadal development in the sand lamprey examined in the present study differed from those of the Japanese river lamprey mainly in that the cystic stage of gonadal development, which was characteristic of the latter species, was obscured, resulting in a hastened sex differentiation of the gonads, and in that the degree of testicular development was quite different in different individuals of a similar size. However, gonadal sexes appeared to have been established prior to the initiation of metamorphosis in the sand lamprey as well as in the Japanese river lamprey. It has been suggested that the life cycle of nonparasitic "satellite" species of lampreys has been curtailed as compared with that of their parasitic partners (cf. Hardisty and Potter, 1971a,b). It was not possible in the present study to discriminate distinct year classes among the collected specimens of

the lamprey. It seems likely, however, that the duration of larval life may be shorter in the nonparasitic sand lamprey than in the parasitic Japanese river lamprey. If this is true, the observed lability of sex differentiation in the sand lamprey would be caused by just such a shortening of larval life, which would serve to modify the relatively stable process of gonadal sex differentiation as observed in the Japanes river lamprey (Fukayama and Takahashi, 1982).

It is well known in lampreys, especially in nonparasitic species, that the sex ratio of adults is generally biased in the male direction (Hardisty and Potter, 1971b; Potter et al., 1974; Malmqvist, 1980). In the present study, ratios of male to female adults of the sand lamprey during the spawning period deviated from normal each year; 1.4 in 1980 and 0.5 in 1981. Some trials to explain this phenomenon in terms of the lability of sex during the larval period have not been successful (Hardisty, 1965; Hardisty and Taylor, 1965). In ammocoetes, after the completion of sex differentiation, males and females are almost equal in number (Hardisty, 1965; Fukayama and Takahashi, 1982). This is true of the sand lamprey observed in the present study. No signs of sex reversal from ovary to testis were observed in the sand lamprey during or after metamorphosis. The observed differences in sex ratios of adult lampreys remain to be explained by further studies.

In the sand lamprey, a rapid maturation occurred in both sexes during the metamorphic period. Vitellogenesis followed by a rapid growth of oocytes commenced in all metamorphosing females, though ovaries of female ammocoetes were invariably in the previtellogenic stage. A remarkably active proliferation of spermatogonia started in all metamorphosing males. Such a conspicuous acceleration of gonadal development during metamorphosis has hitherto been noted in other nonparasitic lampreys as well, such as *Lampetra planeri* (Hardisty, 1965, 1970, 1971), *Mordax praecox* (Hughes and Potter, 1969) and *Ichthyomyzon gagei* (Beamish and Thomas, 1983). Larsen (1980) stated, based on the existence of some neotenic *Lampetra zanandreai* reported by Zanandrea (1956, 1957), that metamorphosis by itself seemed not to be an essential prerequisite for sexual maturation of lampreys. To date, however, no report has dealt with the appearance of neotenic ammocoetes except for those of Zanandrea (1956, 1957), and neotenic ammocoetes were never detected in the sand lamprey of the present study. Accordingly, there remains a possibility that rapid sexual maturation of nonparasitic lampreys may be triggered by a certain physiological change accompanying metamorphosis.

The actual mechanism of metamorphosis in lampreys is quite unknown at present (cf. Hardisty, 1979). Thyroid hormones have been shown to have no essential role in metamorphosis of lampreys (cf. Hardisty, 1979). However, blood levels of thyroid hormones of lampreys change dramatically during the period of metamorphosis. Wright and Youson (1978) revealed that, in the anadromous sea lamprey, *Petromyzon marinus*, the concentration of serum thyroxine (T_4) was very high during the ammocoete stage and declined remarkably during the metamorphic period. Suzuki (1982) also showed that plasma triiodothyronine (T_3) and T_4 of the Japanese river lamprey gradually increased during the ammocoete stage and dropped down dramatically after metamorphosis. More recently, Lintlop and Youson (1983) demonstrated that the serum T_3 levels of the sea lamprey, *Petromyzon marinus*, and the brook lamprey, *Lampetra lamottenii*, were very

high at an initial phase of metamorphosis and significantly lowered afterwards. Also, in preliminary measurements of T_4 and T_3 in the plasma of the sand lamprey (Fukayama et al., unpublished results), levels of thyroid hormones were very high at the end of the ammocoete stage and during the early phase of metamorphosis (T_4, $\geqslant$4.0 μg/100 ml; T_3, 0.32–0.42 μg/100 ml) and declined strikingly thereafter (T_4, 49.5–63.7 ng/100 ml; T_3, $<$0.025 ng/100 ml).

It has been stated repeatedly that thyroid hormones may play some role in sexual maturation of lower vertebrates. For example, Hurlburt (1977) suggested a synergistic stimulatory action of thyroid hormones and gonadotropin on ovaries of the goldfish, *Carassius auratus*. In lampreys, however, no information has thus far been given concerning a possible role of thyroid hormones in the development of gonads. Some experimental studies are now being carried out by the present writers to elucidate this problem.

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