Natural Sex Reversal in Vertebrates

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Natural sex reversal in vertebrates

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Introduction

> With regard to sex, some animals are divided into male and female, but others are not so divided ... In quadrupeds, the duality is universal ... but among the insects and fishes, some cases are found wholly devoid of this duality of sex ... for instance, the eel is neither male nor female, and can engender nothing.
>
> Aristotle

The phenomenon of sex reversal and hermaphroditism in vertebrates has been observed since early human history dating back to Aristotle, who also recorded that the *channa*, a sea-bass, reproduced without copulation. Despite their common occurrence and early discoveries, hermaphroditic animals, with or without sex reversal, aroused fear rather than interest before the eighteenth century; the laying 'cock' and the crowing 'hen' were usually put to death in accordance with the medieval laws (Evans 1906). The first experimental approach to intersexuality and sex reversal in vertebrates commenced with the work on birds and domestic mammals by John Hunter, who once gave an account to the Royal Society of London in 1780 on a most extraordinary pheasant, which 'after having produced several broods, moulted, and the succeeding feathers were those of a cock. This animal was never afterwards impregnated ...' (Marshall 1964).

Sex reversal, the change from one sex to another, is a diversified subject, which, in a broader sense, includes many aspects of intersexuality, hermaphroditism and congenital abnormalities. A brief reference to the basis of sexuality is necessary before the consideration of sex reversal. It is generally realized that the most fundamental attribute of sex is that it brings into being new genetic combinations upon which natural selection may act. The basic nature of sex is represented by the existence of genetic and physiological (biochemical) duality between the mating cells, a phenomenon perhaps best illustrated by the spontaneous chromosome transfer and conjugation in some bacteria (Lederberg 1959), ciliates and flagellates. Such basis of sexuality is described as either the physiological sex (Witschi & Opitz 1963) or the chromosomal sex (Armstrong 1964). In metazoans, there occur invariably specialized organs, the primary sex organs, which are responsible for the production and maturation of the mating cells or gametes. The basis of sexuality hence depends upon the type of primary sex organ present in the organism, so that an individual possessing testes is known as a male and the one with ovaries as a female. This is known as the gonadal sex (Witschi & Opitz 1963; Armstrong 1964). In addition, there exists, particularly among higher vertebrates, the external sex (the sex accessories and the secondary sex characters) and the social-psychological sex (sexual behavioural patterns). In vertebrates, it is possible that sex reversal could have existed in the social-psychological sex or in the external sex without necessarily involving a change in the gonadal sex (Crew 1927). The present discussion will consider only the changes in the primary sex organs in relation with the maturation of gametes and steroid secretion. The change of sex in psychological aspects (such as homosexuality in mammals), or in the plumage sexual coloration in birds, or in the external sex organs in various vertebrates are therefore excluded. On the other hand, among the vertebrates, *pathological* functional sex reversal from female to male involving the primary sex organs has been reported in birds with ovarian tumors (Boring & Pearl 1918; Crew 1923), and *experimental* functional sex reversal could also be induced in the teleost, *Oryzias latipes* (Yamamoto 1953, 1958, 1959, 1961), and in the amphibians, *Pleurodeles waltlii* (Gallien 1950, 1951, 1954) and *Xenopus laevis* (Chang & Witschi 1955, 1956; Gallien 1955, 1956). These are also excluded from the scope of the present discussion on the phenomenon of *natural* sex reversal in vertebrates.

According to the type of primary sex organ present in the organism the sexuality of vertebrates can be briefly classified into two main groups (1) gonochorism, and (2) hermaphroditism.

Gonochorism is the existence of either testes or ovaries in one individual, a condition to which the majority of vertebrates conforms. At maturity each individual functions either as male or as female, despite the usual occurrence of embryonic intersexuality in the gonadal primordia of all tetrapods and elasmobranchs.

Hermaphroditism is the possession of both ovarian and testicular tissues in the same individual which functions both as male and female during its life history. Among the teleosts, in which the expression of sexuality is most complex and diversified, many forms of hermaphroditism are exhibited. Atz (1964) provided a most detailed and comprehensive review on the subject. For simplicity, the present discussion will be restricted to only functional hermaphroditism, i.e. both mature eggs and sperms are produced by a single individual during its life-cycle. In this respect, hermaphroditism can be either (*a*) functional in space, or (*b*) functional in time.

(*a*) *Hermaphroditism functional in space.* Hermaphroditism in which both eggs and sperms mature at the same time in an ovotestis. This is *Synchronous* hermaphrodite (Atz 1964) or *Simultaneous* hermaphrodite (Reinboth 1962).

(*b*) *Hermaphroditism functional in time.* Hermaphroditism in which the individual possesses both testicular or ovarian tissue, but at any one time, functions either as male or as female accordingly. In the normal ontogeny of the gonad, there occurs a complete replacement of the gonadal tissue of one sex by another usually with a transitory intersexual stage. This can be *Protandrous* hermaphrodite, e.g. Platycephalidae (Aoyama, Kitajima & Mizue 1963), in which the ovaries replace the testes, or *Protogynous* hermaphrodite, e.g Maenidae and Synbranchidae (*Monopterus*), in which the sequence is reversed. In vertebrates, natural sex reversal implies the phenomenon of such a sequential expression of male and female phenotypic sexes in the normal gonadal ontogeny of hermaphrodites functional in time. Its occurrence is mainly confined to some teleosts, where the phenomenon is not uncommon, for instance, both protandrous and protogynous forms are commonly found among Sparidae and Serranidae.

The discussion on the sexuality in vertebrates, especially in teleosts, is normally tedious because of the great diversity within the group, and also our present knowledge on hermaphroditism and sex reversal is fragmentary. This report on natural sex reversal in vertebrates serves to provide a simple account on the existing information and views mainly on *Monopterus* and with brief reference to Sparidae and Serranidae. The object is dual. First, to bring to the knowledge of biologists in various disciplines the existence of a less known type of sexuality in which the male and female gonadal sexes are sequentially expressed in relation with ontogney. Secondly, to emphasize that the ontogenetic pattern concerning morphological and physiological functions of the gonad throughout the life-history should be taken into consideration in studies on sexuality and sex determination.

Hermaphroditism in Serranidae, Sparidae and Maenidae

In serranids (the sea-basses, combers and groupers) and sparids, (the porgies and sea-breams) the most diversified expression of sexuality and hermaphroditism is found. Hermaphroditism in these groups consists of synchronous forms such as *Serranus scriba* (van Oordt 1929; D'Ancona 1949; Reinboth 1962), *S. hepatus* (D'Ancona 1949; Reinboth 1962), *S. phoebe* and *Hypoplectus unicolor* (Smith 1959), protogynous hermaphrodites such as *Epinephelus guttatus*, *E. striatus* (Smith 1959), *Pagellus erythrinus* (D'Ancona 1949; Reinboth 1962), protandrous hermaphrodites such as *Sparus auratus* (Pasquali 1941; D'Ancona 1941), *Boops salpa*, *Pagellus acarne* (Reinboth 1962), *P. mormyrus* (D'Ancona 1949), and rudimentary hermaphrodites such as *Sargus annularis*, *S. vulgaris* and *Oblada melanura* (D'Ancona 1949; Reinboth 1962). Some serranids, such as *Roccus* sp., *Paranthias furcifer*, *Rypticus saponaceus* (Smith 1959), and the sparid, *Dentex dentex* (D'Ancona 1949) are gonochorists. In Maenidae, all the three species studied, *Maena smaris*, *M. chrysilis* and *M. maena*, are protogynous (Zei 1949; Lozano Cabo 1953; Lepori 1959; Reinboth 1962).

The basic structures of the gonads of serranids, sparids and maenids are essentially similar. The gonad consists of a paired ovotestes fused posteriorly while the cavities of the two lobes form a common duct. Internally each lobe comprises of a tubular wall with ingrowth of cords, the ovarian lamellae, where ovarian follicles develop and mature. On the ventro-lateral wall of the posterior part of each lobe, testicular tissues are visible as two bands adjacent to the lumen of the gonad. Despite the co-existence of ovarian and testicular tissues in the ovotestes, there is a localization of the sexual areas in most of the serranids, with some exceptions in the groupers and their relatives (Atz 1964). In sparids and maenids the male and female sexual zones are separated by connective tissue. Depending on the type of sexuality and hermaphroditism in the genus or the species, the pattern of gonadal development varies. In the protandrous species, *Sparus auratus*, Pasquali (1941) and D'Ancona (1941) reported that the testicular region of the gonad started to mature during the first year of life-history, and became functional during the second year, after which it regressed. Sex reversal occurred between the age of two to three and the ovarian portion then developed and became functional in the third year, continuing to produce ripe eggs each spawning season for the remaining two years or so of the life-history.

In *Pagellus acarne*, natural sex reversal occurs in the fourth, fifth or sixth years, with the actual structural transformation normally taking place between spawning seasons (Reinboth 1962). In protogynous species, such as the maenids, the ovarian portion matures first while the testicular region remains as a tiny crest adjacent to the gonadal lumen. After two to three spawning seasons, in which the animal functions as a female, sex reversal starts naturally when the ovarian portions degenerate and the testicular part enlarges, occupying the remnants of the ovarian regions; spermatogenesis becomes active and the male portion functional (Lozano Cabo 1953; Lepori 1959; Reinboth 1962). In synchronous hermaphroditic species, e.g. *Serranus scriba*, both male and female regions mature simultaneously, although self-fertilization is possible (Dufosse 1856; Clark 1959; Reinboth 1962; Salekhova 1963), group-spawning is usually observed in natural habitat (Reinboth 1962; Salekhova 1963) and cross-fertilized eggs are reported to survive better than artificially self-fertilized ones (Salekhova 1963).

With regard to natural sex reversal in the protogynous maenids, Lozano Cabo (1953), Lepori (1959) and Reinboth (1962) all agreed that not every individual would undergo sex reversal, an observation suggesting the complexity in sexuality among sex reversing hermaphrodites. It is also interesting to note that most hermaphrodites normally exhibit a co-existence of both male and female sexual areas, a condition whereby a reversal of the gonadal sex is possible by sequential maturation.

Natural sex reversal in *Monopterus*

The occurrence of natural sex reversal in *Monopterus* was first reported by Liu (1944) who established that in field specimens all the small animals were females and all the large ones males, and that intersexual gonads were found only among specimens of medium size. In his review on the sexuality in lower vertebrates, Bullough (1947) discussed the significance of this phenomenon in *Monopterus* in the studies of intersexuality and sex determination in vertebrates. Liem (1963) reported a 4-year study on 898 laboratory-raised specimens of different ages and sizes and also on 96 field specimens from Java, and confirmed the occurrence of natural sex reversal in *Monopterus*. However, no detailed description of histology of the gonad was given in these publications. Chan & Phillips (1967*a*, *b*; Chan, Wright & Phillips 1967) provided a detailed investigation on the structure of the gonad and the general features on the phenomenon of natural sex reversal, with special emphasis on the origin and the localization of the male and female germ-cells and on the pattern of endocrine tissue in both the female and male phases. A simultaneous and independent study on the distribution of the male and female germ-cells was made by Okada (1966). Both Okada (1966) and Chan & Phillips (1967*a*) concluded that the male germ cells pre-existed in the gonad before sex reversal.

Essentially the gonad of *Monopterus* comprises a sterile tubular wall enclosing a spacious gonadal sinus, and two folded structures in which the germinal cells develop and mature. These two folded germinal areas of the gonad was named gonadal lamellae (Chan & Phillips 1967*a*) because both female and male germ-cells develop sequentially in these areas. Most of the specimens of body length less than 35 cm possessed a gonad which was typically ovarian in having developing oocytes and maturing follicles. Animals of body length 35 to 45 cm, at about 2 to 3 years of age, possessed the early intersexual gonad in which the appearance of the male germ-cell clusters were observed as nodules in the gonadal lamellae (figure 1*a*, plate 5). The gonad remained ovarian having mature and normal follicles, and the male germ-cell clusters remain static, comprising mainly spermatogonia and some primary spermatocytes. Sex reversal was normally a post-nuptial event and the development of the male germ-cells was extensive in gonads which exhibited obvious post-ovulatory features (Chan & Phillips 1967*a*). One of the most obvious features during sex reversal was the development of the interstitial Leydig cells in

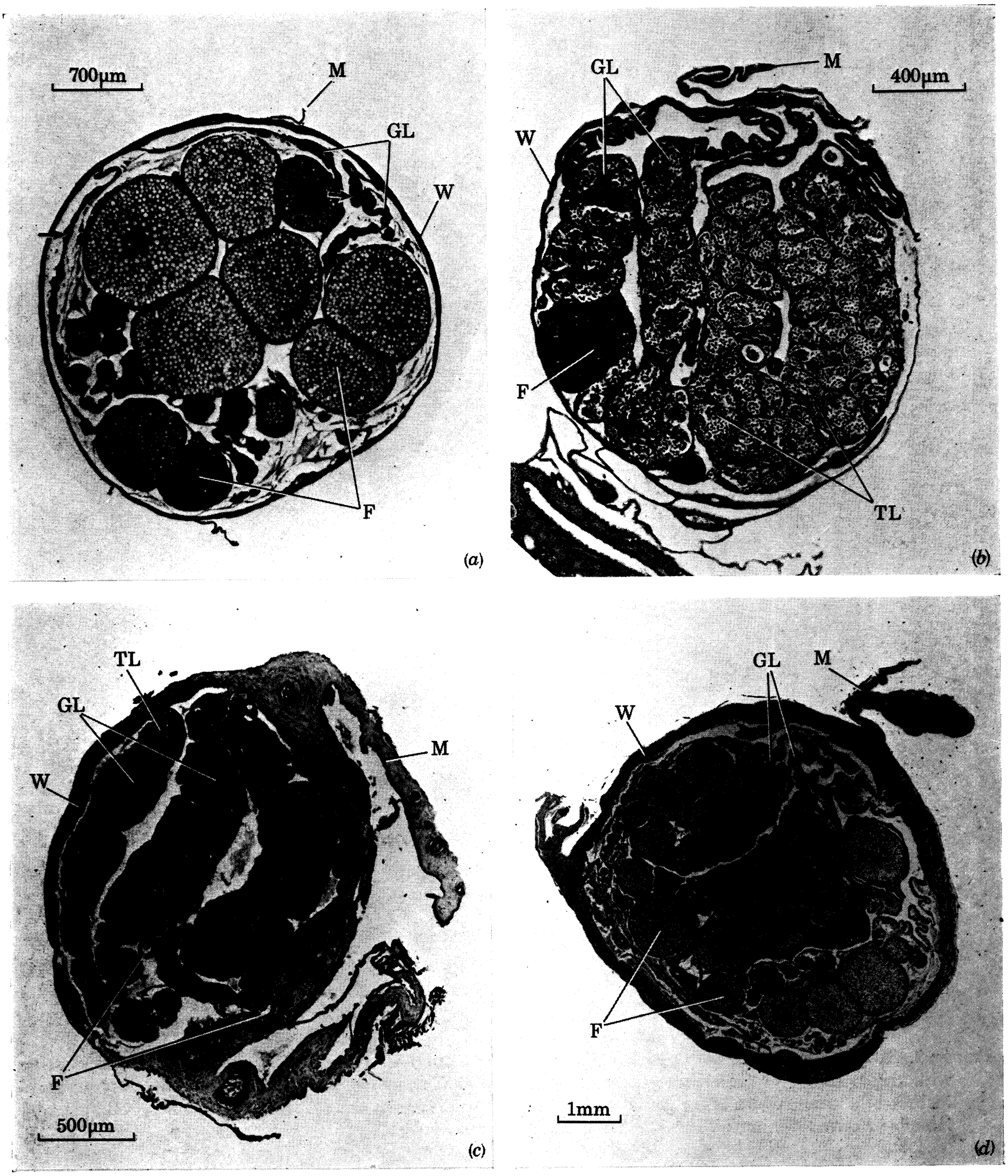

FIGURE 1. Natural sex reversal in *Monopterus*. (*a*) Section of the gonad at early intersexual stage. Essentially ovarian, but male germ-cells develop as clusters (arrows). (*b*) Late intersexual stage. Functionally male, testicular development included the whole width of gonadal lamellae (see also Figure 2). Some remaining follicles indicated a preceding female phase. (*c*) Small male (21 cm). Testicular lobules well developed in the gonadal lamellae, little ovarian tissue, the presence of a functional female phase doubtful. (*d*) Large female (62 cm). Numerous maturing follicles, functionally female. Male germ-cells latent in the inner region of the gonadal lamellae, the presence of a functional male phase doubtful. (F, ovarian follicle; GL, gonadal lamella; M, mesogonium; TL, testicular lobules; W, gonadal wall.)

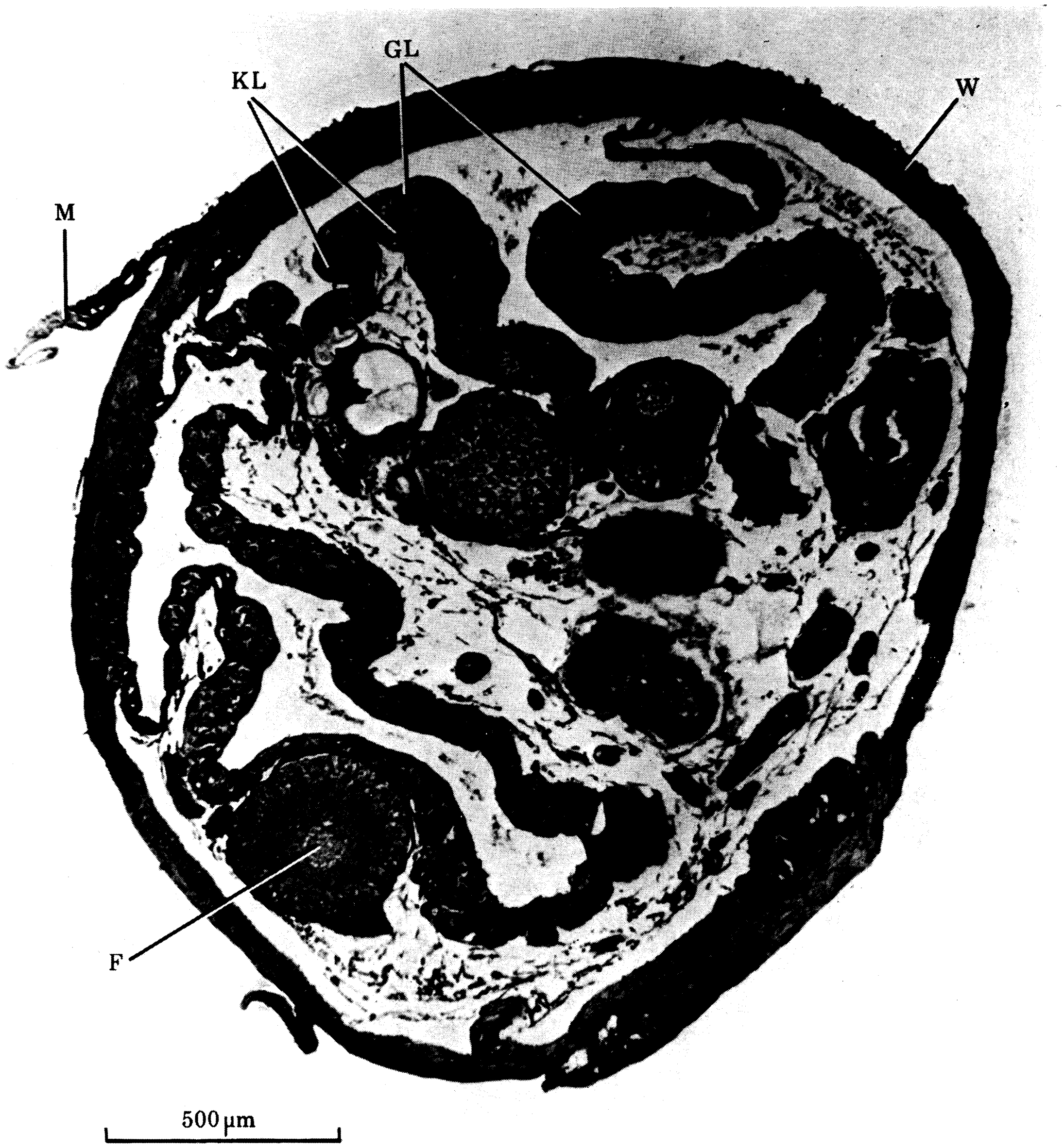

FIGURE 2. Section of gonad at mid-intersexual stage during natural sex reversal in *Monopterus*. A transitory stage with degenerating ovarian tissue, development of testicular lobules and interstitial cells extensive and included the whole width of the gonadal lamella (see also Figure 1*a* and *b*). (F, ovarian follicle; GL, gonadal lamella; M, mesogonium; TL, testicular lobules; W, gonadal wall.)

the interstitium of the gonadal lamellae in concomitance with the proliferation of the male germ-cells which, in the majority of the specimens observed, would occupy the whole width of the gonadal lamellae (figure 2, plate 6). The process of structural transformation usually took 3 to 5 months and by the succeeding breeding season, in late intersexual gonad, a mature testis was observed except for the remnants of a few follicles; the spermatogenetic condition clearly indicated that these were functional males (figure 1*b*). In the gonad of the large, fully mature males, the whole gonadal sinus was nearly filled by the expansion of the gonadal lamellae resulting from active spermatogenesis (Chan & Phillips 1967*a*), however, the folded nature of the gonadal lamellae could still be discerned especially in the post-nuptial period when the testicular lobules regressed and collapsed (Chan & Phillips 1967*b*).

The studies of Liu (1944), Bullough (1947), Liem (1963) and Chan & Phillips (1967*a*) agreed that sex reversal in most of the field specimens occurred in the second or the third year of the life-history. However, Chan & Phillips (1967*a*) provided evidence that some small animals possessed very few ovarian tissue and follicles and that development of testicular tissue had started in a few animals of such a small size that should have normally been female; the overall histological conditions of the gonads of these small animals suggested the female phase was transitory and whether some of these animals have ever functioned as female is doubtful (figure 1*c*). However, as follicles existed, it is likely that the female phase in these animals was short and that sex reversal occurred early in the ontogeny of the gonad. On the other hand, the presence of the large females and large intersexes provided strong evidence for the existence of individuals which changed sex late in the life-history (Chan & Phillips 1967*a*). Evidence derived from observations on the large half-cord intersexes indicated some of the large females of 60 cm long could have changed sex and function as males (see Chan & Phillips 1967*a*). Nevertheless, it is also possible that some individuals might have remained as functional females with the male areas latent in the gonadal lamellae throughout its life-history (figure 1*d*).

It must be emphasized that the difference in size could only be interpreted as a rough indication of age, but body length and sexuality correlation is relevant to a certain degree. Furthermore, the idea of deviation in sexuality, in terms of early or late sex reversal, is supported by the obvious histological difference in gonadal structures and spermatogenetic activities in the male phase of these specimens (Chan & Phillips 1967*a*); the designation of full-cord and half-cord males is arbitrary, simply for the convenience in description (Chan & Phillips 1967*a*). As far as histology of the gonad is concerned, the range of variety observed suggested to be a continuous series of variations, with the majority falling into the normal protogynous hermaphroditic patterns, whereas deviations extended to the condition of stronger male and of stronger female (i.e. with longer male phase or longer female phase in the life-history), a condition best described as a gradation of sexuality.

The investigation on the capacity of steroidogenesis by the gonad during natural sex reversal in *Monopterus* has been studied (Chan & Phillips 1969). Under *in vitro* conditions, *Monopterus* gonads at various sexual stages were capable to convert pregnenolone to androstenedione, testosterone, 17β-oestradiol, and oestrone, although the productions of oestrogens were low in small females and in males (figure 3). Such steroidogenic capacity in *Monopterus* gonad was similar to that in other gonochoristic vertebrates (see Chan & Phillips 1969). Owing to the limitations of analytical techniques and also that the gonad of *Monopterus* was a dynamic and compound organ, there was difficulty in the expression of absolute production rate and in the interpretation of the data. However, given an organ with a dual capacity of secreting both oestrogens and androgens, the essential factor to be considered was the ratio of these two groups of antagonistic hormones. In this respect, the comparison on total production clearly indicated that more oestrogens were produced in the large female and more androgens in the male; the early intersex exhibited a state of balance (figure 3). Furthermore, when the ratio of total androgens to total oestrogens was taken as a quotient, Q_x, to indicate the hormonal maleness, the values suggested an increase of hormonal maleness during the process of sex reversal in both series. The relative low value of Q_x in the half-cord series reflected the comparatively less active spermatogenesis and the more female nature in the histological observations of their gonads.

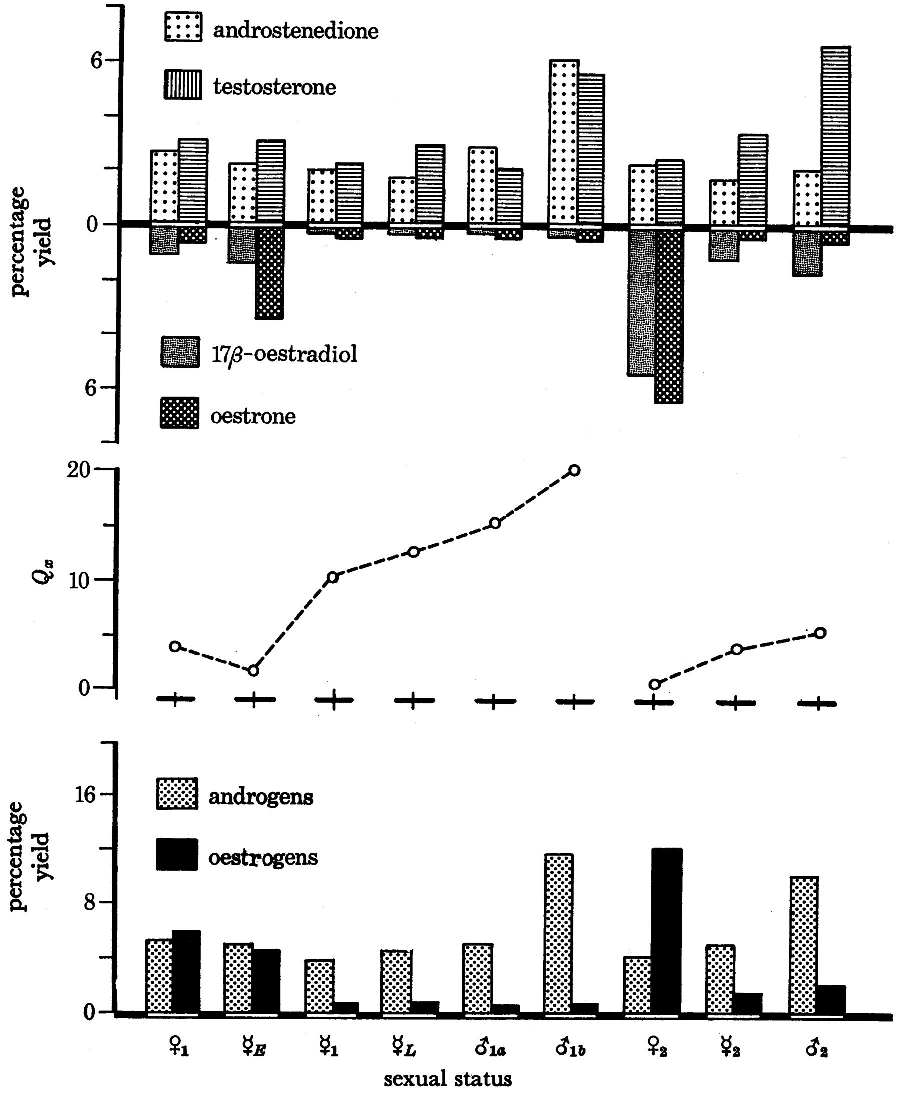

FIGURE 3. *In vitro* steroidogenesis in *Monopterus*. $♀_1$ (small females), $☿_E$ (early intersexes), $☿_I$ (mid-intersexes), $☿_L$ (late intersexes), $♂_{1a}$ (small males), $♂_{1b}$ (large males), $♀_2$ (large females), $☿_2$ ($\frac{1}{2}$-cord intersexes), $♂_2$ ($\frac{1}{2}$-cord males). Q_x (androstenedione + testosterone)/(17β-oestradiol + oestrone)

The increase in secretion of androgen was apparently associated with the development of interstitial Leydig cells during the start of testicular development, but whether the onset of androgen secretion was primary or secondary to the switch mechanism of natural sex reversal in *Monopterus* remains unknown. However, it is clear that the structural transformation in the gonads from ovary to testis is accompanied by a shift in the endocrine secretion from female to male; a correlation between the structural and physiological functions therefore exists during natural sex reversal in *Monopterus*.

Hermaphroditism and evolution

Little is known about the evolution of hermaphroditism in vertebrates, and the phylogeny of functional hermaphroditism in teleosts, with or without sex reversal, remains an unsolved problem. According to D. E. Rosen (see Atz 1964), a synchronous hermaphrodite should have a reproductive potential nearly twice that of a gonochorist with the same individual energy requirements. However, the obvious evolutionary disadvantage is probably the possibility of self-fertilization and inbreeding in synchronous hermaphroditic forms, though the group-spawning behaviours in *Serranus scriba* in natural habitats (Reinboth 1962; Salekhova 1963) might possibly provide a situation in increasing the chances for cross-fertilization. On the other hand, as far as prevention of self-fertilization is concerned, the sequential expression of male and female phases in separate spawning seasons among the protogynous and protandrous hermaphrodites could have provided a mechanism as good as in gonochorists.

With regard to the phylogenetic position of hermaphroditism in the evolution of heterosexuality, van Oordt (1929), D'Ancona (1949, 1950, 1952) and Smith (1959) considered that, in serranids and sparids, the synchronous species are primitive, from which protogynous, protandrous and gonochoristic forms may have evolved. The diversified expression of sexuality in sparids seemingly provided a step-wise progress from synchronous forms to protogynous or protandrous species, and through rudimentary hermaphroditism of various forms and frequency to species of structural and functional gonochorists. And some rudimentary hermaphroditic individuals were found in species normally gonochoristic, a condition believed to be evidence for a hermaphroditic ancestry for these gonochoristic sparids (D'Ancona 1949). Liu (1944) and Bullough (1947) also suggested that protogynous hermaphroditism such as in *Monopterus* is primitive. Schriener (1956) proposed a similar view for the evolution of gonochorism from hermaphroditism in *Myxine* and *Ephetretus*.

The alternative view is that hermaphroditism in the families Labridae, Synbranchidae, Cyprinodolidae and the Order Myctophiformes represents various lines of independent evolution (see Atz 1964) and that functional hermaphrodites in these groups are secondarily evolved from gonochoristic forms (Stéphan 1902; Harrington 1961; Atz 1964; Liem 1968). Harrington (1961) reported that *Rivulus marmoratus* were usually found singly or in small numbers in isolated and limited bodies of water, and that the capacity, and the normal occurrence, of self-fertilization in the tooth-carp provided strong evidence that these synchronous hermaphroditic forms were derived from gonochoristic females under the selective pressure of severe isolation (see Atz 1964). There is little solid explanation on the evolutionary process leading to a complete replacement of gonochoristic forms by hermaphrodites which originated from gonochoristic females. The idea of Pelseneer (1894) was cited by Atz (1964) that: (1) production of spermatozoa in the ovaries of some females; (2) reduction in size and number of males; (3) replacement of all females by hermaphroditic forms, and (4) disappearance of the degraded males. Based on a similar idea, Liem (1968) postulated that under the selective pressure of seasonal droughts in its natural habitat, sex reversal in *Monopterus* could have evolved, so that the females, by sex reversal, could give rise to the transformed males (secondary males);

animals with bilobed testes were reported as evidence for the existence of the original gonochoristic males (primary males) in which no sex reversal occurred (Liem 1968). The equation of primary males to full-cord males of Chan & Phillips (1967*a*) and secondary males to half-cord males was also claimed (Liem 1968). Such comparison seemed hazardous as there was obvious evidence that natural sex reversal had occurred in most of, if not all, the full-cord males (Chan & Phillips 1967*a*).

The selective value of self-fertilizing capacity of *R. marmoratus* in the greatly isolated habitat is undoubtedly significant (Harrington 1961; see Atz 1964). However, in *Monopterus*, the selective pressure of isolation should be considered in the light of the following points. First, that the seasonal droughts in the tropics normally occur only in the winter months does not favour a regular existence of isolation pressure during April to September (the prenuptial and the spawning periods) to result in reproductive isolation. Secondly, the prenuptial migratory behaviour (Wu & Liu 1942) does not support the idea of severe reproductive isolation in *Monopterus*. Furthermore, it is difficult to see how the sexuality of a protogynous hermaphrodite as *Monopterus* having its female and male phase structurally and functionally expressed in separate spawning seasons (e.g. second year a female and third year a male) could have any greater selective advantage over, and replaced, the gonochoristic forms under a yearly isolation pressure.

The evolution of natural sex reversal in *Monopterus* remains an open question. As far as gonadal structure is concerned, the variations in ontogenetic pattern of female and male phase suggested the existence of a gradation of sexuality. The occurrence of individuals of stronger male and stronger female need not necessarily be a representation of an evolutionary progress of hermaphroditism to gonochorism in vertebrates, although a similar trend might have had occurred in the ancient vertebrate stock (see Bullough 1947). The condition in *Monopterus* may possibly be a range of variations deviated from the normal protogynous hermaphroditic pattern. These variations in the ontogenetic expression on the duality of sex (e.g. a longer female phase with a shorter male phase, or vice versa) might be phylogenetically primitive or selectively neutral and non-adaptive so that the species might remain 'polymorphic' in sexuality, unless the non-adaptive changes of drift bring about that one character is fixed and the other suppressed. It should be recalled that the most fundamental attribute of sex is that it brings into being new genetic combinations upon which natural selection may act, and that as far as cross-fertilization is concerned, the sex reversing pattern, such as in *Monopterus*, could probably provide a mechanism as effective as in gonochoristic species.

Sex determination and natural sex reversal

Two comparative aspects should be included in the discussion of the determination of sexuality in vertebrates: (1) genetic basis for sex determination; (2) somatic organization of gonad and inductor substances.

Genetic basis for sex determination

In vertebrates, the existence of a genetic sex-determining mechanism has been established by studies on sex ratio, sex linkage, breeding of sex-reversed animals such as *Oryzias latipes* (Yamamoto 1955, 1959), and by the actual identification of chromosomal difference between the sexes (heterogamety and homogamety). The concept of 'chromosomal determination of sex' is based upon the recognition that sexuality is dependent on the difference in sex chromosomes among mammals, birds and reptiles (Beatty 1964). However, in lower vertebrates, the differentiation of sex chromatin does not seem to have been reported (Dodd 1960, 1964; Beatty 1964). Genetic evidence for the existence of sex chromosomes is available only for a limited number of species of fishes which have been extensively studied by Winge, Aida and Yamamoto. But the work of Winge on *Lebistes* (1934), Aida (1936) and Yamamoto on *Oryzias* (1953, 1955, 1958, 1959, 1961, 1962, 1965) and Witschi on *Xenopus* (1943, 1942, 1957) demonstrated that, among these lower vertebrates having a heterogametic–homogametic sex mechanism, not only experimental alteration of sex chromosome into autosome and vice versa appeared possible (Winge 1934), but also the reversal of phenotypic sex disharmonious with the normal genotypic sex could be produced by selective breeding. The interpretation that genetic sex determination is a genic balance rather than merely chromosomal difference is widely held (Bridges 1932; Witschi 1934, 1960; Winge 1934; Crew 1952; Gordon 1957; Dodd 1960; Gowen 1961; Yamamoto 1962).

The conceptual idea of a genetic mechanism in sex determination, as Witschi first discussed the problem in 1934 on amphibians, is that the primary sex determination rests upon the balance between the potency (M) of the male-determining genes and the potency (F) of the female-determining genes in the genome; when one potency is in excess of the other heterosexuality would exist even in the absence of heterosexual chromosomes. It is believed that the most primitive genetic mechanism for sex determination is hermaphroditic in nature (Witschi 1934) and in the hypothetical condition, the sex-determining genes are distributed throughout the chromosomes. The evolution of sex chromosomes is supposed to be a result of, first, a congregation of the female-determining genes in one particular autosome, which then becomes the X chromosome, and then followed by quantitative changes in the female genes, either diminution in the male line or augmentation in the female line, resulting in the formation of X(F) and Y(f) chromosomes (Witschi 1934; Crew 1952; Gordon 1957; Burns 1961; Forbes 1961; Gowen 1961). It follows that in a normal homogametic female, (FF) in the two X chromosomes is always in excess of (MM) in the autosomes; in the normal male, (MM) is more potent than (Ff) in X and Y chromosome. In intersexes and hermaphrodites, potencies of the male and female determining genes are equal (see also Crew 1952).

Little is known about the genetic constitution and its control on sex determination in teleosts where intersexes, functional hermaphroditism and sex reversals are common. It is generally accepted that a genetic basis for sexuality exists in all teleosts and such a genetic mechanism is postulated to be primitive, labile and multifactoral (Winge 1934; Witschi 1942; Bullough 1947; D'Ancona 1949, 1960; Crew 1952; Dodd 1955, 1960; Hoar 1955, 1965; Gordon 1957; Bertin 1958; Burns 1961; Gowen 1961; Forbes 1961; Lagler, Bardack & Miller 1962; Yamamoto 1962).

For protogynous species with sex reversal, Liu (1944) and Bullough (1947) denied the presence of genetic sex-determining mechanism in *Monopterus*, whereas Atz (1964) maintained that it was difficult to see how a protogynous hermaphrodite such as *Monopterus* could be in any way less genetically governed than gonochoristic species. If sexuality is so expressed that one individual is either a male or a female only, and that the chromosomal mechanism of XY (or ZZ) and XX (or ZW) determines male and female respectively, it would seem that such mechanism is absent in functional hermaphrodites whose phenotypic sex is neither only male nor only female, but both. However, as every trait must have its genetic background and the adult phenotype is the result of a series of developmental steps, a structure should not be defined as the form at a single stage of the life-history. Rather it should be represented by the whole sequence of forms that make up the ontogeny; more accurately, it should not be defined only as the forms but rather as the sequence of the series of changes that underlie the change of forms. It would seem that the determination of sex, i.e. organogenesis of the gonad and subsequent events concerning the maturation of the germ-cells, is governed by a system of multiple factors of relative male and female tendency, which, in whole, controls the sequential events in gonadal ontogeny and its physiological function throughout the life-history.

In vertebrates, as far as the primary sex organs and the production of germ-cells are concerned, three patterns exist in the expression of sexuality in relation with ontogeny. Each of these represents a form of expression in sexuality governed by its sex determination system of various degree in the developmental homeostasis between the male and female sexuality.

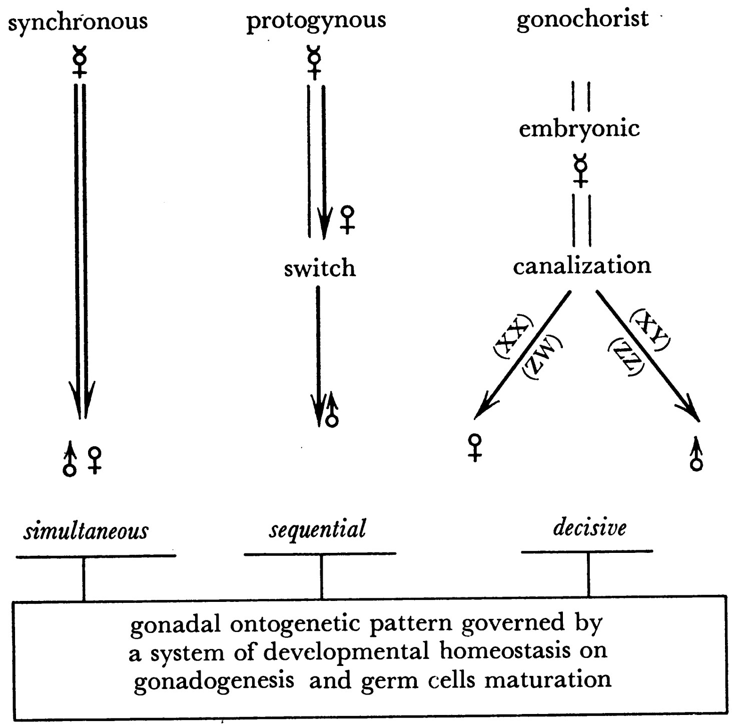

Figure 4. Patterns of sexuality in relation with ontogeny of the gonad.

(1) In synchronous hermaphrodites, the relative male and female tendency in the system is expressed in parallel, and the simultaneous development and maturation of both gametes is possible, probably in the absence of a switch-mechanism.

(2) In gonochorists, though the embryonic intersexuality in tetrapods and elasmobranchs offers a bipotential capacity in the development and expression of both sexes, however, the system for developmental homeostasis is decisive towards one sex whereby a canalization in gonadal ontogeny is resulted by, first, the total suppression on the development of one sex area, and secondly, a definite induction on the developmental pattern of the other, resulting in the phenotypic expression of one sex only. In gonochoristic teleosts, Chavin & Gordon (1951), Gordon (1952) and Öktay (1959) have demonstrated, by crossing various male and female strains of platyfish, the existence of both a system of relative male and female potency and a switch mechanism for either male or female development; the condition can be either homologous or parallel to that in tetrapods.

(3) In sex reversing hermaphrodites, the relative male and female potency in the system is narrow or labile resulting only in the temporary suppression of one sexual part without complete destruction. In the normal ontogeny of a protogynous hermaphrodite, the developmental pattern is always the female first until a switch mechanism operates, either by the decline or removal of the female part or by direct stimulation and spontaneous maturation of the male part, shifting the development to the latent one and hence resulting in natural sex reversal. The change in the relative male and female tendency in the system would change, in this respect, not the protogynous pattern of the gonadal ontogeny, which should be species specific, but the duration in the sequential expression of the two alternative sexes, although the ontogenetic stage at which the change occurred would impose some influence on the degree of expression of the succeeding sex (see Chan & Phillips 1967*a*).

Somatic organization of gonad and sex inductors

In tetrapods and elasmobranchs, the occurrence of a double sex anlage and its antagonistic nature in the embryonic gonad have been accounted for the rarity in functional hermaphroditism and in natural sex reversal among these groups. In teleosts, there appears no such uniform pattern in somatic organization; in salmonids, cyprinids, cyprinodonts and some others, the whole genital ridge originates from the peritoneal wall and is therefore homologous to the cortex of amphibians and amniotes (D'Ancona 1949, 1950, 1952; Dodd 1964). However, the gonads of Maenidae, Sparidae and Serranidae possess a separate localization of male and female sexual areas in a common substratum, D'Ancona (1949) and Yamamoto (1962) considered this condition was different from that of other gonochoristic teleosts and apparently similar to the amphibians, although the association of the male portion of the ovotestis with the interrenal blastema has not been confirmed (D'Ancona 1949). Nevertheless, the localization of male and female sex areas in hermaphrodites such as *Monopterus* provides a structural duality of sex whereby natural sex reversal could have been expressed by sequential maturation of the two areas.

Because of the lack of experimental and embryological information for the fishes, it is not fully known whether a sex inductor system exists. Chieffi (1959), working on *Torpedo ocellata* and *Scylliorhinus canicula*, established by embryological investigation and by steroid administration that the mechanism of inductor-antagonism probably operated in elasmobranch fishes. In teleosts, our knowledge has not advanced beyond hypothetical ideas. D'Ancona (1949) proposed that, in the hermaphroditic sparids and serranide, the two different germ areas of the gonad produced two distinct sex-differentiators, the *gynogenine* and the *androgenine*. Yamamoto working on *Oryzias latipes*, a gonochorist in which functional sex reversal can be experimentally induced, also suggested the existence of the genic induced inductor-substance, the *gynotermone* and the *androtermone*, as an antagonistic system for sex differentiation in teleosts (Yamamoto 1962; Yamamoto & Matsuda 1963). In addition, the view that steroid androgens may act as 'andro-inductors' and oestrogens as 'gyno-inductors' in fishes was also put forward (Yamamoto 1962).

In amphibians and teleosts, it is well known that sex steroids may act as controlling factor in the postgenetic sex determination superimposed on the primary genetic sex determination. Wolff (1962) suggested that steroid hormones were the key substances in sex differentiation in vertebrates and that the embryonic inductor-substances are steroids modulated by protein and lipids, while Dodd (1964) suggested that sex inductors were steroid–protein complexes. On the other hand, Chieffi (1965) reported a survey on the onset of steroidogenesis in the vertebrate embryonic gonads by histochemical techniques and concluded that sex inductor-substances in elasmobranchs, amphibians, reptiles and mammals were probably different from the adult sex steroids. In teleosts, Hishida (1965) demonstrated the conversion of oestrone to 17β-oestradiol in the gonad of *Orzyias latipes* during the period critical for sex differentiation, a fact suggesting that the embryonic gonad metabolized steroid hormones.

Whether embryonic inductors are related or homologous to adult sex steroids remains a problem for further investigation. It is clear that the study on the relationship of gonadal ontogeny, both embryonic and post-embryonic development, and developmental endocrinology, including the nature of embryonic sex inductors and the onset of steroidogenesis in the gonadal primordia, becomes increasingly important. Owing to their special ontogenetic patterns in the gonads, the hermaphroditic teleosts would therefore be of special significance in comparative endocrinology and reproductive physiology in vertebrates.

Natural sex reversal in protogynous hermaphrodites such as *Monopterus* is unique among the vertebrates in their capacity to function sequentially both as female and as male during its life-cycle. The functional shift, both structurally and physiologically, from one sex to another during the natural ontogeny in the gonad of *Monopterus* (Chan & Phillips 1967*a*, *b*, 1969) suggests that the protandrous and protogynous hermaphrodites are excellent material for the study of endocrinological and ontogenetical relationships. Besides, as it has been realized by other workers such as Bullough (1947) and D'Ancona (1949), that the various types of sex reversal might represent a similar condition in the ancient vertebrate stock both in genetic constitution and in gonadal organization, the studies on the natural sex reversal and intersexuality in lower vertebrates might perhaps provide some basic knowledge to the unsolved problems in sex determination, such as the endocrine induction of sexuality and the evolution of heterogametic–homogametic sex mechanism in vertebrates.

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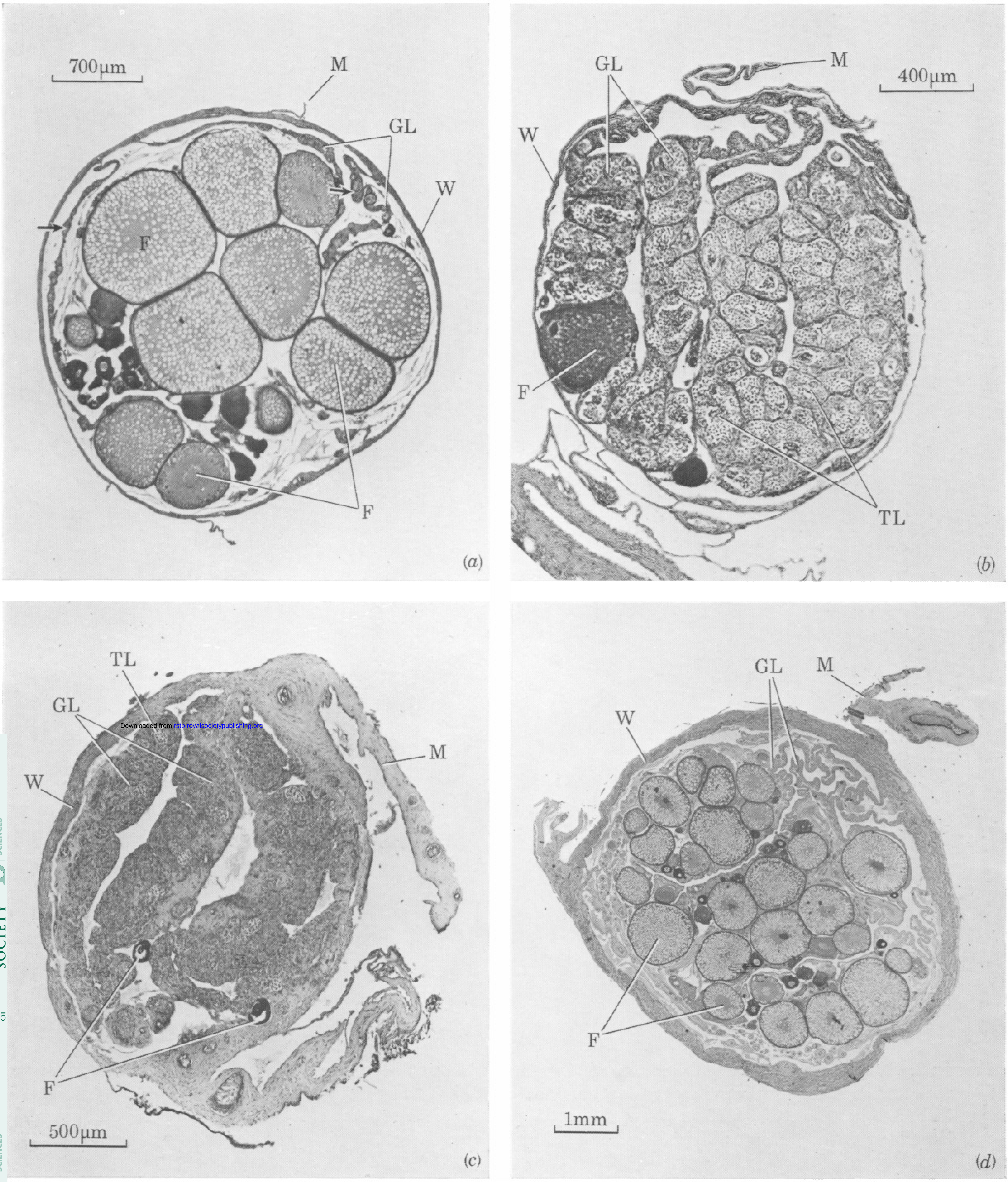

FIGURE 1. Natural sex reversal in *Monopterus*. (*a*) Section of the gonad at early intersexual stage. Essentially ovarian, but male germ-cells develop as clusters (arrows). (*b*) Late intersexual stage. Functionally male, testicular development included the whole width of gonadal lamellae (see also Figure 2). Some remaining follicles indicated a preceding female phase. (*c*) Small male (21 cm). Testicular lobules well developed in the gonadal lamellae, little ovarian tissue, the presence of a functional female phase doubtful. (*d*) Large female (62 cm). Numerous maturing follicles, functionally female. Male germ-cells latent in the inner region of the gonadal lamellae, the presence of a functional male phase doubtful. (F, ovarian follicle; GL, gonadal lamella; M, mesogonium; TL, testicular lobules; W, gonadal wall.)

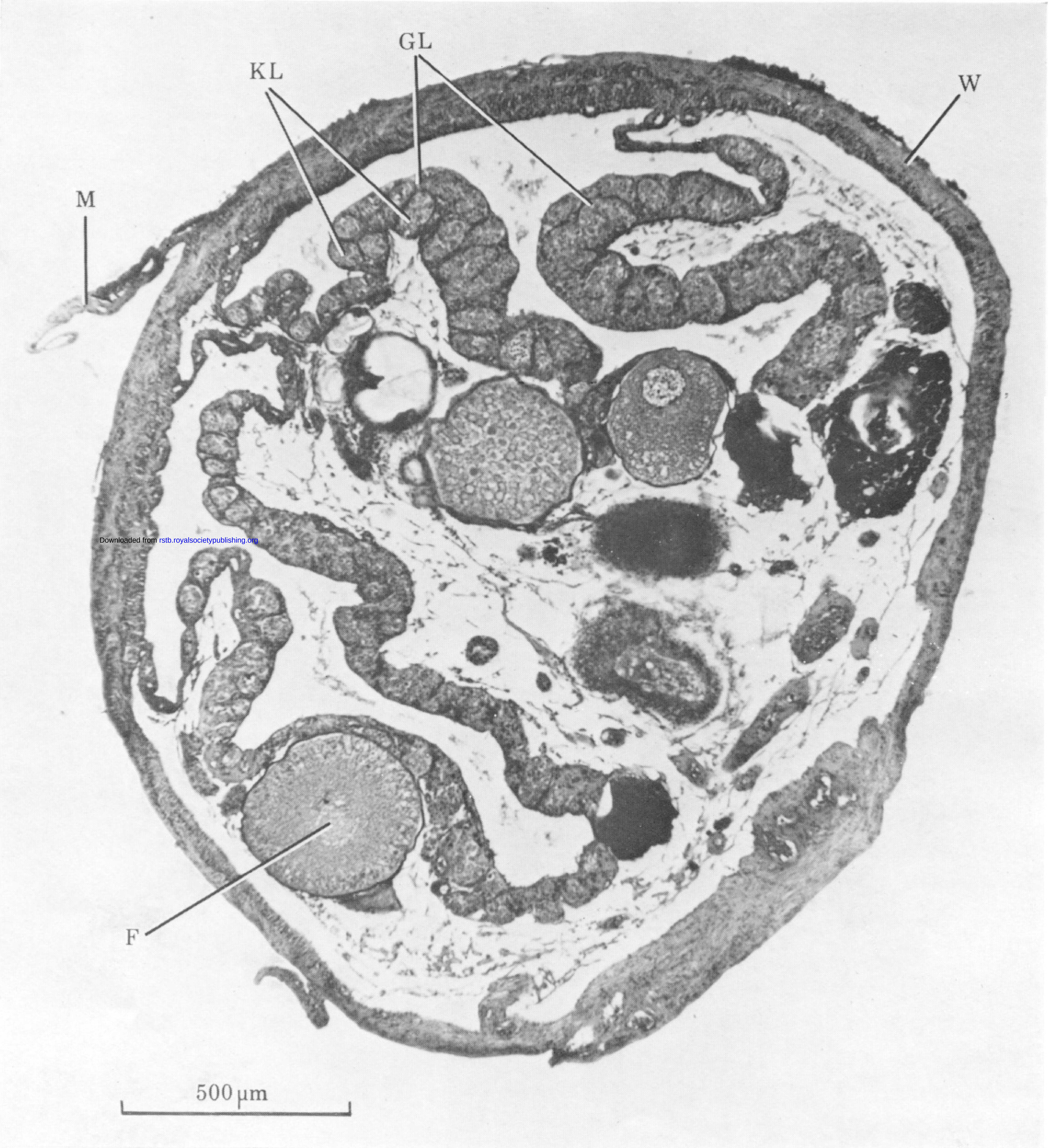

FIGURE 2. Section of gonad at mid-intersexual stage during natural sex reversal in *Monopterus*. A transitory stage with degenerating ovarian tissue, development of testicular lobules and interstitial cells extensive and included the whole width of the gonadal lamella (see also Figure 1 *a* and *b*). (F, ovarian follicle; GL, gonadal lamella; M, mesogonium; TL, testicular lobules; W, gonadal wall.)