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Review article

Sex determination and sex reversal: genotype, phenotype, dogma and semantics

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Summary. The genetic terminology of sex determination and sex differentiation is examined in relation to its underlying biological basis. On the assumption that the function of the testis is to produce hormones and spermatozoa, the hypothesis of a single Y-chromosomal testis-determining gene with a dominant effect is shown to run counter to the following observed facts: a lowering in testosterone levels and an increase in the incidence of undescended testes, in addition to sterility, in males with multiple X chromosomes; abnormalities of the testes in autosomal trisomies; phenotypic abnormalities of XX males apparently increasing with decreasing amounts of Y-chromosomal material; the occurrence of patients with gonadal dysgenesis and XY males with ambiguous genitalia in the same sibship; the occurrence of identical *SRY* mutations in patients with gonadal dysgenesis and fertile males in the same pedigree; and the development of XY female and hermaphrodite mice having the same genetic constitution. The role of X inactivation in the production of males, females and hermaphrodites in T(X;16)16H mice has previously been suggested but not unequivocally demonstrated; moreover, X inactivation cannot account for the observed bilateral asymmetry of gonadal differentiation in XY hermaphrodites in humans and mice. There is evidence for a delay in development of the supporting cells in XY mice with ovarian formation. Once testicular differentiation and male hormone secretion have begun, other Y-chromosomal genes are required to maintain spermatogenesis and to complete spermiogenesis, but these genes do not function effectively in the presence of more than one X chromosome. The impairment of spermatogenesis by many other chromosome abnormalities seems to be more severe than that of oogenesis. It is concluded that the notion of a single testis-determining gene being responsible for male sex differentiation lacks biological validity, and that the genotype of a functional, i.e. fertile, male differs from that of a functional female by the presence of multiple Y-chromosomal genes in association with but a single X chromosome. Male sex differentiation in XY individuals can be further impaired by a euploid, but inappropriate, genetic background. The genes involved in testis development may function as growth regulators in the tissues in which they are active.

Introduction

"There is no such biological entity as sex. What exists in nature is a dimorphism within species into male and female individuals, which differ with respect to contrasting characters, for each of which in any given species we recognize a male form and a female form, whether these characters be classed as of the biological, or psychological, or social orders. Sex is not a force that produces these contrasts, it is merely a name for our total impression of the differences" (Lillie 1932).

"The male-determining action of the human Y chromosome could be effected in either of two ways. It could, itself, be the bearer of the genetic information necessary for the development of testes (and consequently masculine genitalia), or this information could be located on one or more different chromosomes and normally demand the presence of a Y chromosome for activation. According to the first of these alternatives the presence of a Y chromosome (or of a particular segment of a Y chromosome) would be essential for masculine development. The second requires that the Y chromosome should bear a locus (or loci) that acts as an activator (or repressor) of another locus or loci: it leaves open the possibility that, exceptionally, activation might be brought about in other ways. According to this hypothesis the presence of a Y chromosome would normally be necessary, but not essential, for masculine development" (Ford 1970).

"... the discovery of an equivalent zinc finger protein gene (now designated *ZFX*)... required some adjustment of the previously accepted dogma... that a Y-linked, testis-determining gene would operate as a single dominant factor, whose presence, or absence, alone would determine sex" (Craig 1990).

"... two independent but complementary studies both support the heretical notion that the male-determining

action of the *Y* in marsupials is not absolute" (Graves 1990).

The above quotations illustrate that, during the past 60 years, there has been a shift in scientific perception that has crystallized into a "dogma" that all the multiple differences that distinguish male and female mammals can be traced back to the presence or absence of a single gene. What is the biological basis on which this dogma was founded?

The Oxford English Dictionary defines a male as "belonging to the sex which begets offspring, or performs the fecundating function of generation", whereas "female" means "belonging to the sex which bears the offspring". It is evident, however, that both classes contain a subset of individuals that are unable to fulfil these functions. Biologically, indeed, there is an entire spectrum of phenotypic manifestations that bridges the gap between the sex that begets the offspring and the sex that bears it (Polani 1970); this necessarily introduces a degree of arbitrariness into the binary male/female classification. Its applies to a large proportion of the individuals, both human and mice, who feature in discussions on the genetics of sex determination, as many such individuals are unable to reproduce and exhibit the contrasting characters that we recognize as male and female (Lillie 1932) to varying degrees. The purpose of the present review is to sharpen the arguments in this subject by examining the relationship between the genetic terminology and its underlying biological basis.

Sex determination and differentiation

These widely used terms are given different meanings by different investigators. "Sex determination" is the process that results in either testis or ovary formation, whereas "sex differentiation" is the process subsequent to gonad formation, according to Goodfellow and Darling (1988); an essentially similar definition is adopted by Erickson et al. (1987). In contrast, McCarrey and Abbott (1979) define "sex determination" in a more abstract sense, as the event of genetic programming, and subdivide the actual processes of development into "primary sex differentiation", i.e. the development of the gonad, and "secondary sex differentiation", i.e. the development of accessory sex organs, e.g. ducts and genitalia.

An intermediate position is taken by Eicher and Washburn (1986), who describe the process leading to the development of fetal gonads into testes or ovaries as either "primary (gonadal) sex determination", or as "primary sex differentiation", whereas the development of the Müllerian or Wolffian duct system comes under the heading of "secondary sex differentiation".

We see that the process resulting in the differentiation of the gonadal ridge into ovary or testis is variously referred to as "sex determination", "primary sex differentiation", or "primary (gonadal) sex determination". The genetic terminology underlying this process reflects the twin paradigms of mammalian sex differentiation: (1) the role of the fetal testis in masculinizing the reproductive tract *vis-à-vis* the dispensability of the fetal ovary for the purpose of feminizing the reproductive tract (Jost 1947, 1970), and (2) the wide-spread concurrence of the presence of a Y chromosome with male phenotype, and the absence of Y with female phenotype. On the basis of these considerations, and largely ignoring the second alternative set out by (Ford 1970) (which allows for multiple loci on the Y chromosome normally, but not invariably, being necessary for male development), a hypothesis has recently gained ground that the Y chromosome contains a single "testis-determining" gene, named *TDF* (*Tdy* in the mouse). It is sometimes referred to as a "switch" gene (McLaren 1988).

The position of the hypothetical gene on the short arm of the Y chromosome was deduced by correlating deleted Y chromosomes with their phenotypes (Jacobs and Ross 1966; Davis 1981). A candidate gene, *ZFY*, which is located at a distance of 140 kb from the pseudoautosomal region, and which was isolated by Page et al. (1987), fell out of favour with the discovery of a number of facts thought to be incompatible with the characteristics of a "testis-determining" gene. Chief among these was the finding of four patients, three XX males and one true hermaphrodite, who carried Y-derived DNA sequences excluding *ZFY* on one of their X chromosomes (Palmer et al. 1989); the authors argued that, although the phenotypes of XX males lacking Y-derived DNA sequences could be explained by downstream mutations, exchange of Y-specific sequences next to the pseudoautosomal boundary redefines the region containing *TDF*. Subsequently, the *SRY* gene was isolated from a 35-kb region on the human Y chromosome (Sinclair et al. 1990), and a corresponding sequence, *Sry*, was cloned from the mouse Y chromosome (Gubbay et al. 1990). This gene, on a 14-kb genomic fragment, was found to induce testicular development in 3 out of 11 transgenic XX mice (Koopman et al. 1991), thus strengthening the claim that *SRY* is *TDF*. However, what exactly does this statement mean?

Since a basic tenet in present-day discussions is the dominant effect of the mammalian Y chromosome on male sex determination (Koopman et al. 1991), it is instructive to examine the biological basis underlying this argument in the context of Klinefelter's syndrome and its variants.

Testis development in males with XXY karyotypes

Most patients with Klinefelter's syndrome are diagnosed after puberty, but careful examination reveals abnormalities at much earlier ages. In two series of 47,XXY neonates ascertained at birth, birth weights were found to be lower than in 46,XY controls (Ratcliffe et al. 1979; Robinson et al. 1979). Taking the two series together, 6 out of 65 newborn XXY infants had undescended testes. Ratcliffe (1982) gives the incidence of incomplete descent of one or both testes at the birth of boys with XXY Klinefelter's syndrome as 6.3%, compared with 0.87% in XY controls. In a selected sample of 170 adult married patients examined by Kleczkowska et al. (1988), cryptorchidism was found to be present in 20.

Wheras small testis size with absent spermatogenesis is accepted as a practically invariant characteristic of 47,XXY Klinefelter's syndrome, relatively little attention has been paid to testicular development in prepubertal boys. Ferguson-Smith (1959) wrote that he suspected testicular length to be reduced in chromatin-positive boys with Klinefelter's syndrome, but he was unable to back this up with comparable data on normal boys. This paper was published in *The Lancet* on 31st January 1959, the same date that saw the publication, by Jacobs and Strong (1959), of the XXY chromosome constitution in Klinefelter's syndrome. Henceforth, cytogenetics became the dominant field of investigation, whereas clinical findings attracted less interest. The discovery that the presence of a Y chromosome accompanied by more than one X chromosome was associated with a male phenotype gave rise to the idea of the dominant effect of the human Y chromosome on testis development, with little attention being paid by geneticists to the nature and size of the resulting testis.

Notwithstanding statements to the contrary, it has been established that the testes in Klinefelter's syndrome show abnormalities long before puberty. Ferguson-Smith (1959) found a large reduction in the number of spermatogonia in mentally retarded, chromatin-positive boys aged 7–12 years, and a similar effect was reported by Mikamo et al. (1968) in three infants of 12 months or less. Indeed, the study by Coerdt et al. (1985) demonstrates that germ cells are abnormal before birth. A series of six 47,XXY fetuses, of crown-heel length 19–28 cm, showed an average reduction of germ cells per tubular section of about 60% compared with XY fetuses, whereas a second investigation of five mid-term XXY fetuses, involving the use of semi-thin sections, gave a percentage-germ-cell-per-testis volume of 9.45, compared with 19.6% for XY testes.

Although these studies did not provide any evidence of early maldevelopment of somatic cells in XXY testes, underdevelopment of genitalia occurs in a significant minority of prepubertal boys. In the Cytogenetics Centre in Leuven, hypogonadism was the major indication for karyotyping in 13 out of 40 boys, in whom the 47,XXY constitution was established between birth and 12 years of age (Kleczkovska et al. 1988). Among a sample of 18 boys ascertained as a result of the Edinburgh chromosome survey, Ratcliffe et al. (1979) found slow growth of the penis in 4 individuals.

That somatic abnormalities are also increased in 47,XXY patients is evident from the study by Robinson et al. (1979), summarizing findings on 63 infants. The incidence of major abnormalities was 18%, and that of minor abnormalities 26%, compared with, respectively, 1% and 7% in the patients sibs. Major abnormalities in XXY children included cleft palate, inguinal hernia, and kidney agenesis.

In adult patients, Leydig cells are abnormal, and mean testosterone levels are about half those of normal males.

Males with more than two X chromosomes

It became apparent 30 years ago that the abnormalities seen in XXY Klinefelter's syndrome are exacerbated in patients with 49,XXXXY chromosomes. In an early review, Fraser et al. (1961) wrote that "Of eight testes only one (in Miller et al.'s case) is descended". In describing the fifth patient with this chromosome anomaly, Fraccaro et al. (1962) wrote: "it is conceivable that testes are present but undescended and if so, the results of endocrine determination are strongly suggestive of impairment of their function". These patients also had moderate to severe mental retardation and abnormalities of ossification.

In a more recent review, Fryns et al. (1983) write: "The 49,XXXXY karyotype constitutes a recognizable clinical syndrome. In contrast to Klinefelter's syndrome, the external genitalia are hypoplastic from birth on, with a small penis and underdeveloped scrotum ('mini-male external genitalia'). Bilateral cryptorchidism is frequent; in other cases, soft testes may be felt in the inguinal canals". Severe pathological changes are seen in prepubertal testes. Evidently, additional numbers of supernumerary X chromosomes exacerbate the pathological changes associated with the presence of a single supernumerary X, and abnormalities, which occur in a minority of 47,XXY patients, are the norm in those with 49,XXXXY chromosomes, and frequently occur with increased severity. Leydig cells may be entirely absent (de Grouchy and Turleau 1982; Fryns et al. 1983), and major malformations and severe mental retardation have also been recorded (de Grouchy and Turleau 1982).

In summary, the "dominant" effect of the Y chromosome on male sexual development is attenuated in the presence of supernumerary X chromosomes, resulting in sterility and less frequent hypogonadism. In virtually all cases, the germ cells die and the genetic fitness is zero.

The incomplete dominance of the Y chromosome in ensuring the development of a functional male is even more pronounced in marsupials. It seems appropriate, therefore, to include a brief account of XXY marsupials.

XXY intersexes in marsupials

Sharman et al. (1990) described two wallabies, one belonging to the species *Macropus eugenii*, the other a member of *M. rufus*, with XXY chromosomes. Both had abdominal abnormal testes, a pouch, and no scrotum. A third individual, an eastern grey kangaroo, *M. gigantus,* which was a mosaic with XXY/XX/XY chromosomes, had the same phenotype, and another individual of this species with XXY chromosomes appeared to be similar, but was still being investigated. According to Shaw et al. (1990), a single X chromosome results in the development of a scrotum, whereas two X chromosomes give rise to a pouch.

In an introduction to the above reports, Graves (1990) cites these findings as evidence that the sexual phenotypes of aneuploid marsupials depends not only on the presence or absence of a Y chromosome, but also on the number of X chromosomes present. As was shown in the preceding paragraphs, this phenomenon is not confined to marsupials. Moreover, the number of autosomes present may also affect the sexual phenotype.

The testis in autosomal trisomies

The study by Coerdt et al. (1983) of fetal testes with anomalies included seven fetuses with trisomy 13 and six each with trisomy 21 and 18. In the cases with trisomy 13 or 21, the average proportion of germ cell volumes, when compared with those of 46,XY fetuses, was reduced by about 50%, similar to the level observed in 47,XXY fetuses, whereas the reduction was greater in trisomy 18. It is noteworthy that, in a single 47,XYY fetus, the shortfall was less than in any other trisomy. A reduction in germ cell numbers also occurs in female fetuses with autosomal trisomies, as shown by Cuniff et al. (1991).

Testicular volumes were shown to be reduced (on average by 40%–50%) in 35 males with Down's syndrome aged 18–35 years when compared with control patients in the same hospial (Sylvester and Rundle 1962). Two of the 35 Down's syndrome patients had undescended testes, compared with 3 cases out of 88 in the mentally retarded control group. The incident of nondescent appears to be higher than in the normal male population.

Sperm counts in Down's syndrome patients were carried out by Stearns et al. (1960) and were found to be in the oligospermic range. Johannisson et al. (1983) studied testicular histology and synaptonemal complexes in one patient. Histological changes corresponded to spermatogenic arrest, thought to be caused by the association of an unpaired chromosome 21 with the XY bivalent. Evidence that many chromosome anomalies may result in impairment of gametogenesis prior to pachytene has been presented by Mittwoch and Mahadevaiah (1991). There are two substantiated accounts of a male patient with Down's syndrome who initiated a pregnancy (Sheridan et al. 1989) and fathered a child (Bobrow et al. 1992).

It is evident that there is considerable overlap between the pathological manifestations of the testis in trisomies caused either by an extra chromosome 21 or by an additional X chromosome, with the latter condition being associated with more severe abnormalities of the testis. In male patients with trisomies 13 and 18, cryptorchidism seems to be the rule. Therefore, not all the abnormalities seen in Klinefelter' syndrome are specific for disomy of the X chromosome or trisomy of the pseudoautosomal region. Disomy of the X chromosome is also a feature of XX males, who, however, differ from XXY males in not being trisomic for the pseudoautosomal region, and in lacking at least the major part of the Y chromosome. These patients were, until recently, regarded as cases of "sex reversal" and have played a particularly important role in endeavours to elucidate the genetic basis of male development. Their phenotypic manifestations will now be considered.

XX males

The first patient regarded as being an XX male had bilateral testes and ambiguous genitalia (Shah et al. 1961). In his review of 45 patients, de la Chapelle (1972) wrote that "Taken together, the anatomy of the genitalia in XX males is apparently indistinguishable from that of subjects with XXY Klinefelter's syndrome". However, retention of one or two testes occurred in 6 out of 35 cases, and hypospadias was reported in 4 individuals. Furthermore, patients exhibiting more pronounced intersexual phenotypes were excluded from the classification "XX male", e.g. an infant with ambiguous genitalia, bilateral testes and XX chromosomes, described by Cleveland and Chang (1965), and the patient reported by Duck et al. (1975), who presented as a girl aged 14 years with cliteromegaly and increasing signs of masculinization, and who had bilateral testes and well-developed Müllerian structures.

The average height of XX males described by de la Chapelle (1972) was less than in XXY and XY males but greater than in XX females. There seems to be less mental retardation than in XXY patients, since XX males are rarely found in hospitals for the mentally retarded.

The application of recombinant DNA technology has shown that XX males can be divided into two classes: those who carry Y-derived DNA sequences on one of their X chromosomes, and others who apparently lack Y-chromosomal DNA (Guellaen et al. 1984; de la Chapelle 1987; Ferguson-Smith et al. 1990; Abbas et al. 1990). Ferguson-Smith et al. (1990) give clinical details of 15 XX males, including 10 with Y-chromosomal DNA sequences and 5 with no such sequences. In all 10 patients with Y-sequences, the external genitalia were classified as "normal"; 4 of the Y-negative patients had hypospadias, whereas the fifth was said to have a small penis. Abbas et al. (1990) describe two XX patients who were Y-positive and had unambiguously male phenotypes (although one had a short penis), and 4 Y-negative patients with genital ambiguities. De la Chapelle (1987) suggested the subdivision of patients into two classes: (1) *XX males* and (2) *XX males with ambiguous genitalia and/or hypospadias.* In the first category, two scrotal testes should be present and histologically verified, a criterion that, however, cannot often be met in practice.

The classification of XX males into two classes according to the presence or absence of Y-chromosomal DNA also presents difficulties, since the amount of Y-specific sequences is extremely variable (Ferguson-Smith 1991). In the majority of Y-positive XX males, the Y-chromosomal segment probably spans over 200 kb and includes the *ZFY* gene (Page et al. 1987). Palmer et al. (1989) reported three XX males and one true hermaphrodite (the latter a sib of one of the males) with only about 60 kb of Y-chromosomal material, which included *SRY* but which excluded *ZFY*. In spite of the presence of *SRY*, the degree of masculinization appeared to be no greater than in Y-negative patients, with three of the patients having hypospadias and the third cryptorchidism. A second true hermaphrodite who was Y-positive but *ZFY*-negative has recently been described (Jäger et al. 1990b).

A connection between the XX male condition and true hermaphroditism is suggested by the fact that the two conditions sometimes co-exist in the same pedigree (Skordis et al. 1987; Ostrer et al. 1989; Abbas et al. 1990; Palmer et al. 1989; Pereira et al. 1991). Bradbury (1987) has provided evidence that the testes of XX↔XY chimeras develop from fetal ovotestes, in which the ovarian portion subsequently regresses. In human individu-

als, likewise, an insufficient stimulus for complete testicular differentiation may result in some ovarian development in the fetus, followed by different degrees of regression of the ovarian portion.

It is evident that the concept of the XX male as an individual with normal male phenotype and spermatogenic breakdown has shifted in the direction of intersexuality. To some extent, the phenotype manifestations of these patients are delimited not so much by objective clinical criteria, as by the decision of clinical geneticists regarding which patients should, or should not, be included in this category.

In summary, XX males are not a natural clinical entity, but may be regarded as a subset of intersexual individuals who are not overtly hermaphrodite and whose phenotypes are nearer the male than the female distribution. There is evidence that the phenotype is affected both by the amount of chromosomal material from Yp that is present and by the extent of the deletion in Xp (Ferguson-Smith 1991); however, these correlations are not absolute.

Sxr mice and X-inactivation

The cytogenetic basis of seemingly sex-reversed XX *Sxr* mice can be seen as a dark-staining body of Y-chromosomal origin on their paternal X chromosome (Evans et al. 1982). These mice are usually described as otherwise normal males with small testes devoid of germ cells, although it should be remembered that the clinical manifestations of hypogonadism are probably less exactly defined and recorded in mice than in humans.

Since the Y-chromosomal sequence responsible for testis development is carried on an X chromosome in these mice, the question arises as to whether it might be subject to X-inactivation. Cattanach et al. (1982) concluded that either inactivation did not take place, or the proportion in which inactivation occurred was not sufficiently large for ovarian development to occur.

A different situation is seen in mice that are heterozygous for the T(X;16)16H(T16) X-autosome translocation. T16/X*Sxr* mice exhibit a whole range of sexual phenotypes from sterile male, via hermaphrodite, to fertile female (Cattanach et al. 1982; McLaren and Monk 1982); this has been interpreted as resulting from non-random X-inactivation. In female mice heterozygous for the T16 translocation, the translocated X is almost invariably active, whereas the normal X chromosome is inactivated (Lyon et al. 1964). Therefore, if the latter carries *Sxr*, it might be expected to be inactivated in the large majority, if not all, cells; this could explain the female phenotype. The development of hermaphrodites and males has been explained on the assumption of varying degrees of inactivation of *Sxr*, even though it is carried on an inactive X chromosome. Other explanations, however cannot, so far, be excluded.

Ohno (1985) and Cattanach (1987) have suggested that ovarian development may be the result of the T16 translocation rather than of X-inactivation. The T16 translocation causes growth retardation in female carriers (Lyon et al. 1964; Searle et al. 1983). If ovarian development in T16/*Sxr* mice were entirely caused by X-inactivation, one would expect all T16/X*Sxr* mice to be male, since *Sxr* is carried on the translocated X chromosome, which is active. The relevant data have not yet been published, however, and so the verdict "not proven" is in order regarding whether ovarian development in T16/X*Sxr* mice is caused by the translocation, by X-inactivation, or perhaps by a combination of both factors.

Bilateral asymmetry

X-inactivation cannot explain the biased asymmetry described by Ward et al. (1987), who found that most hermaphrodite T16/X*Sxr* mice had a testis on the left side and an ovary on the right. This bias, moreover, is not confined to T16/X*Sxr* hermaphrodites, since it has also been described in hermaphrodite mice with a *domesticus*-type Y chromosome on backgrounds of different inbred strains, most of which had a left testis and a right ovotestis (Biddle et al. 1991); although among the hermaphrodite mice described by Eicher and Washburn (1983), ovotestes were the predominant gonad, the combination left testis/right ovotestis occurred more frequently than the reverse combination.

The bias of testicular differentiation in favour of the left side in hermaphrodite mice is in contrast to the situation in human hermaphrodites, in whom testicular tissue is preferentially found on the right, and ovarian tissue on the left side, irrespective of the sex chromosome constitution (Simpson 1978; van Niekirk and Retief 1981; Gilgenkrantz 1987), a rather striking confirmation of the statement ascribed to the Greek philosopher Parmenides (5th century BC) "males on the right, females on the left" (Lesky 1950; Lloyd 1973). Evidently, this relationship is reversed in mice.

In searching for an explanation of the opposite asymmetry relationship in humans and mice, it may be rele-

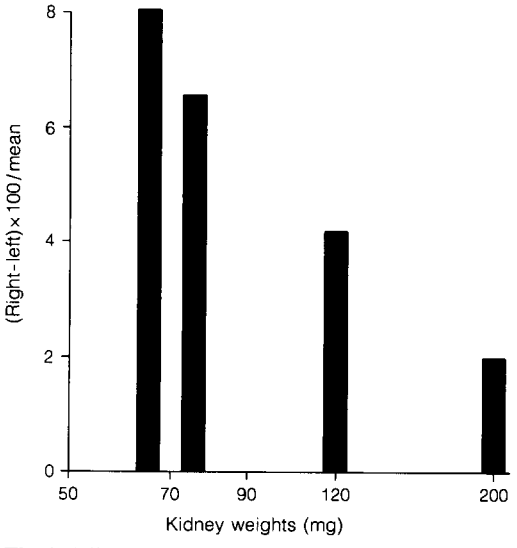

Fig. 1. Bilateral asymmetry in favour of the right kidney in mice decreases with increasing kidney weight. Seven wild population of mice were divided into two groups according to increasing kidney weight, and each group was further subdivided into younger and older mice (data from Mittwoch 1979)

vant to point out evidence that the bilateral asymmetry of kidney weights changes direction with increasing size of the organ (Mittwoch 1979) (Fig. 1). A sample of 7 populations of wild mice, collected by Professor R. J. Berry, were divided into 3 populations with lower kidney weights and 4 populations with higher kidney weights; all populations were further classified into younger and older mice. It was found that right kidneys were heavier than those on the left throughout, but that the difference decreased with increasing kidney weights.

Data by Mackey and Mackey (1938) show not only that there is a similar trend in rats, i.e. that the excess weight of the right kidney decreases with increasing kidney weight, but also that, in line with the difference in kidney size between the two species, the overall asymmetry in favour of the right kidney is less than in mice. In guinea pigs, there is no significant difference between left and right kidneys in males, whereas in females, the left kidney is heavier (Shirley 1976). A similar situation was found in human fetuses. In male fetuses, there was no significant difference between the two kidneys, where as in females, the asymmetry was in favour of the left (Mittwoch and Mahadevaiah 1980). Many years ago, Jackson (1909) wrote that the larger size of the left kidney in humans is established in the early fetal period.

Measurements of human fetal gonads, carried out by Mittwoch and Mahadevaiah (1980) showed a bias in favour of the right gonad; this was more pronounced for ovaries. In fetal mice, Mittwoch and Buehr (1973) were unable to detect a consistent difference between left and right gonads. It may be worth noting, however, that on the basis of an intensive investigation of gonadal growth in fetal rats, Lindh (1961) concluded that "the frequency for the volumen predominance of the left gonad in relation to that of the right is probably not chance". In adult mice, the right testis is the heavier (Hunt 1986; L. A. Setterfield and U. Mittwoch, unpublished). The possibility that there may be a reversal in asymmetry with increasing size of the gonad is suggested by recent evidence that, in embryos of the opossum *Monodelphis domestica* in which the gonads are very small, the bias is in favour of the left gonad (P. J. Baker and U. Mittwoch, unpublished).

These data could provide an explanation for the observed reversal in the bilateral asymmetry of gonadal differentiation in humans and mice. Mice are small animals with a fast rate of development, and it may be expected that the differentiation of their gonads becomes stabilized at an early stage, when the left gonad is still ahead of the right, thus leading to the preferred development of testes on the left. In humans, on the other hand, with a much slower rate of development, the differentiation of the testis and ovary occurs at a later stage, when the right gonad is in the ascendency and, therefore, testicular differentiation is biased in favour of the right.

If bilateral asymmetry of gonadal differentiation in hermaphrodites has its origin in the basic asymmetry of fetal gonads, one would expect the phenomenon to be apparent in XY and in XX hermaphrodites; this is, indeed, observed. The preferential location of testicular tissue on the right occurs in human hermaphrodites with XX, XY and XX/XY sex chromosomes (Simpson 1978; Gilgenkrantz 1987), whereas in hermaphrodite mice, testicular tissue is preferentially situated on the left in T16/X*Sxr* (Ward et al. 1987) and in XY karyotypes (Eicher and Washburn 1986; Biddle et al. 1991). This provides substantial evidence for the contention that true hermaphroditism arises from local variation in genotypes with unstable sex-determining mechanisms (Mittwoch and Kirk 1975; Mittwoch 1986).

Intersex pigs exhibit the same right bias for testicular tissue (Breeuwsma 1970) as in humans, whereas no bilateral asymmetry has been seen in hermaphrodite cocker spaniel dogs (Meyers-Wallen and Patterson 1988).

Nonrandom distribution of ovarian and testicular tissue in ovotestes

The involvement of local factors in gonadal differentiation is further supported by the nonrandom distribution of testicular and ovarian tissue in ovotestes. In 80% of human ovotestes, the two types of tissue are arranged end-to-end, whereas in the remaining 20%, the testicular tissue is situated in the hilar region (van Niekirk 1974). End-to-end association of the two tissues is also characteristic of ovotestes in pigs (Breeuwsma 1970).

In ovotestes of mice, the craniad and/or caudad regions are ovarian, whereas the central region is testicular. This has been shown in mosaics, either XO/XY or XO/XY/XYY (Eicher et al. 1980), and in XX↔XY chimeras (Bradbury 1987).

The triple sequence, ovarian/testicular/ovarian, is reminiscent of the ovotestes obtained by Chang and Witschi (1956) in *Xenopus laevis*, bearing in mind the reversal in heterogamety (Mittwoch 1983) (Fig. 2). Treatment of genetically male, ZZ larvae of *Xenopus* with oestradiol resulted in the gonads developing into ovaries; for complete ovarian differentiation, the hormone had to be administered for about 3 days beginning with embryonic stage 26. If treatment began at stage 26 but was continued for only 2 days, the upper (craniad) gonomeres developed into ovarian tissue, whereas the lower (caudad) portion was unaffected by the treatment and became testicular tissue. If, on the other hand, treatment was delayed until stage 27, the lower gonomeres become ovarian lobes, whereas treatment that started late and finish-

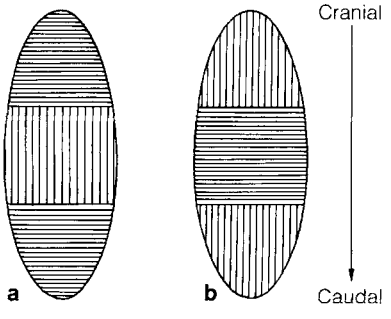

Fig. 2. Triple sequence of ovarian *(horizontal stripes)* and testicular *(vertical stripes)* portions in ovotestes of **a** mice (Eicher et al. 1980), and **b** *Xenopus laevis* (Chang and Witschi 1956). Ovotestes in *X. laevis* were obtained by treating ZZ larvae with oestradiol for a period that was insufficient to induce complete ovarian differentiation

ed early resulted in the triple sequences of testicular/ovarian/testicular ovotestis. By equating insufficiency of the exogenous agent in *Xenopus* with insufficiency of the genetic determinant in mosaic and chimaeric mice, the distribution of ovarian and testicular tissue in mouse ovotestes can be derived, bearing in mind the reversal in asymmetry between *Xenopus* and mammals. The position of ovarian tissue in the anterior (cephalad) part of the ovotestis is equivalent to a delay in testicular differentiation, whereas ovarian tissue at the posterior (caudad) end can be regarded as tantamount to early cessation of the action of the genetic determinant.

XY gonadal dysgenesis and related conditions

Patients with XY gonadal dysgenesis have streak gonads, primary amenorrhoea, and do not undergo puberty (German 1970). They tend to be taller than XX females, and they lack the stigmata of Turner's syndrome. The gonads are at risk of developing gonadoblastomas (Ferguson-Smith 1991). Pedigrees with more than one affected member have been described on a number of occasions (Simpson 1976). In addition, several sibships are known containing a patient with XY gonadal dysgenesis and an XY male with ambiguous genitalia (Simpson 1976). The relationship between XY gonadal dysgenesis and testicular insufficiency is further indicated by a patient who had ambiguous genitalia, and one streak and one contralateral "bean-sized" gonad (Fraccaro et al. 1966). Some pedigrees of XY gonadal dysgenesis show evidence of X-linkage (Simpson et al. 1981; Mann et al. 1983), suggesting a possible common genetic basis with XY females in other mammals, such as the woodlemming, *Myopus schisticolor* (Fredga 1988).

Berta et al. (1990) carried out DNA sequencing of the *SRY* gene in 11 XY female patients and found a *de novo* point mutation in one. A second patient had a point mutation that she had inherited from her father, but that was not found in 50 control males. Another investigation of 14 XY females by Jäger et al. (1990a; 1991) resulted in the identifaction of one *de novo* frame shift mutation, whereas a second point mutation in *SRY* was also present in the patient's father, a brother and an uncle. Similarly, McElreavey et al. (1991) report a family in which a single base change in *SRY* is carried in two generations, including males and patients with pure gonadal dysgenesis. Pivnick et al. (1991) found one mutation in *SRY* among 14 patients with gonadal dysgenesis. These findings provide further evidence that *SRY* is implicated in testicular development, and suggest that a *de novo* mutation is responsible for its failure in a minority of female XY patients, whereas *SRY* appears to be intact in the majority. It is possible that mutations in *SRY* that are inherited from the father may predispose to testicular failure, although still being compatible with normal development in other individuals. It has, of course, not been established whether *de novo* mutations identified in patients with gonadal dysgenesis would lead to testicular failure in all individuals carrying them. This seems to be the case in mice with a deletion including *Sry* (Lovell-Badge and Robertson 1990).

XY female and hermaphrodite mice

In the mouse, a number of different conditions are known that regularly give rise to XY females and hermaphrodites. Eicher et al. (1982) were the first to show that the Y chromosome from mice of the *Mus domesticus poschiavinus* subgroup is incapable of inducing complete testicular differentiation when present on the genetic background of the inbred C57BL/6J (B6) strain. The F1 generation was not affected but the results of backcrossing the wild-derived Y^{POS} chromosome onto B6 suggested the existence of an autosomal gene concerned with testis-determination; this gene was named *Tda-1* (Eicher and Washburn 1986). The authors postulated that this gene exists in different allelic forms, and that these differ in B6 mice compared with the *Mus domesticus* group; another allelic difference is thought to distinguish the Y-linked testis-determining gene, *Tdy*, in the two groups.

Washburn and Eicher (1983, 1989) identified another autosomal locus involved in testis development; this is located in the *T*-locus region of chromosome 17 and was named *Tas* for "T-associated sex reversal". The *Tas* gene is thought to be deleted in the T^{hp} and T^{Orl} deletions, and these, when on a B6 background and in conjunction with an AKR Y chromosome, give rise to XY females and hermaphrodites. The Y chromosome of AKR, like Y^{POS}, is derived from *Mus domesticus* (Bishop et al. 1985). Certain other Y chromosomes of *Mus domesticus* origin giving rise to ovotestes in backcrosses to B6 have since been identified (Biddle et al. 1991).

Eicher and Washburn (1986) have explained their findings by postulating a testis-determining pathway, in which a number of genes are required to act in sequence. The first gene is *Tdy*, and this is followed by a so-far unknown number of autosomal testis-determining genes. In addition, an ovary-determining pathway is postulated, consisting of an unknown number of hypothetical "ovary-determining" genes. In order to effect testicular differentiation, *Tdy* is required to act before the initiation of the ovary-determining pathway, so as to pre-empt, and possibly inactivate, the first ovary-determining gene. In other words, the ovary-determining pathway shadows the testis-determining pathway at a later stage in development.

In this scheme, the concept of the "testis-determining gene" has been replaced by a series of such genes, with *Tdy* being the first; whereas there is no suggestion of the way in which any of the genes in the series might function, it is essential that *Tdy* must act early, since late action by the gene carries the risk of ovarian development. Cattanach (1987) and Biddle et al. (1991) have questioned the necessity for postulating the existence of a discreet gene involved in *T*-associated sex several *(Tas)*, since the T^{hp} and T^{Orl} deletions may themselves be responsible for developmental delay, and hence late action of *Tdy*. This implies that the differentiation of ovarian tissue may not require the mediation of ovary-specific genes, but could be achieved by merely slowing down the rate of development. Therefore, the same genes, or other determinants, could direct the development of testis and ovary, genes causing fast gonadal development promoting testicular

differentiation, and genes causing slow gonadal development giving rise to ovarian differentiation.

Evidence of a developmental delay in genetic combinations that are prone to the formation of XY ovotestes comes from the finding by Washburn and Eicher (1989) that mice carrying an AKR Y chromosome on a B6 background, but without a *Tas* deletion, develop testes with delayed cord formation in the polar regions, i.e. in the same areas that are liable to ovarian differentiation in the genesis of ovotestes; Palmer and Burgoyne (1991) report that testis development is delayed in the presence of the Y^{POS} chromosome. The authors interpret their finding by assuming "a timing mismatch, in which *Tdy* activity is too late, rather than too little, thus failing to pre-empt ovary determination" (Burgoyne and Palmer 1991). However, as shown above, late action can be equated with insufficient action. If a delay in development causes ovarian differentiation, a late-acting testis-determing gene is self-contradictory; it would, indeed, be identical with an ovary-determining gene.

Developmental asynchrony

In order to be translated into morphological or biochemical differences, changes in rates of development in hitherto homogeneous cell types must occur at different rates in tissues that have differentiated previously. The phenomenon of developmental asynchrony has recently been discussed by Haig (1991) in connection with environmental sex determination: temperature determines sex in alligators because if affects the developmental rate of some tissues more than others. In the loggerhead turtle, *Caretta caretta*, Harry and Williams (1991) found a significant difference in the weights of the urinogenital systems at male- and female-determining temperatures: this was not reflected in the over-all weights of the embryos.

For the development of the mammalian testis, there is general agreement that a subset of the somatic cells of the gonad, the so-called supporting cells (McLaren 1991), play a leading role in initiating the formation of sex cords, and so their early growth would seem to be a necessary requirement (Mittwoch 1986, 1989). It is less clear what other tissue might be involved, against which "early" growth could be measured. Theoretically, this tissue could be either the interstitial cells of the gonad, or the non-gonadal cells. Germ cells would seem to be excluded, in view of extensive evidence that they do not play an active role in the initial process of ovarian or testicular differentition (McCoshen 1983; McLaren 1984). The adrenals would seem to be a possibility, since they develop in close proximity to the gonads and secrete potentially active substances, which might interact with gonadal cells.

Whatever the exact mechanism, we may conclude that, for testes to develop, the supporting cells of the gonad need to be ready to form Sertoli cells and sex cords by the time the embryo has reached a particular milestone. Once this time is passed, uncommited supporting cells can no longer give rise to Sertoli cells, sex cords cannot be formed, and the germ cells enter meiosis. The differentiation of the mammalian ovary is thus seen as the default developmental process, and the postulate of specific ovary-determining genes becomes unnecessary. For autosomal genes affecting gonad development, testis determination and ovary determination are two sides of the same coin: fast development will promote the development of the testis, and slow development promotes ovarian differentiation. Y-chromosomal genes will normally preside over the differentiation of the testis, but cannot always guarantee their development.

What do sex-determining genes do?

A model illustrating a threshold dichotomy of testicular differentiation in two species with different developmental rates is shown in Fig. 3. It is assumed that, in order to develop into a testis, a gonad needs to attain a developmental threshold by a given time. The developmental threshold is assumed to be the same in both types of species, but the temporal threshold will be more extended in the species with a slower rate of development. A testis, therefore, needs to develop faster in the faster developing species. It follows that an XY gonad (c in Fig. 3) of the slowly developing species will become a testis within its own developmental background, but if it were to develop at the same speed in a fast developmental background, it will have missed the threshold and become an ovotestis or ovary. The fact that the human *SRY* gene apparently fails to effect testicular development in the mouse (Koopman et al. 1991) is consistent with this hypothesis, since the human gene would be expected to act more slowly than that of the mouse.

An example illustrating the spurt of gonadal growth in relation to that of the fetus as whole is shown in Fig. 4,

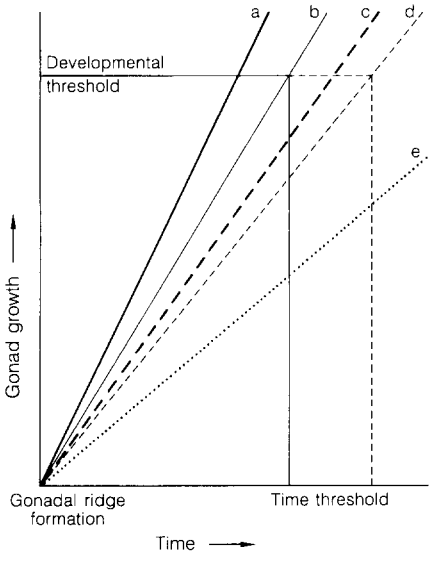

Fig. 3. Model illustrating temporal and developmental thresholds for testicular differentiation in fast-growing *(solid lines)* and slow-growing *(broken lines)* species. *Line a* growth rate of testis in fast-developing species; *line b* theoretical threshold dividing the growth rates of testes and ovaries in fast-developing species; *line c* growth rate of testis of slowly developing species; *line d* theoretical threshold dividing the growth rates of testes and ovaries in slowly developing species; *line e* growth rate of ovary (either species). A gonad whose growth rate is close to the threshold is liable to become an ovotestis or a "sex-reversed" gonad

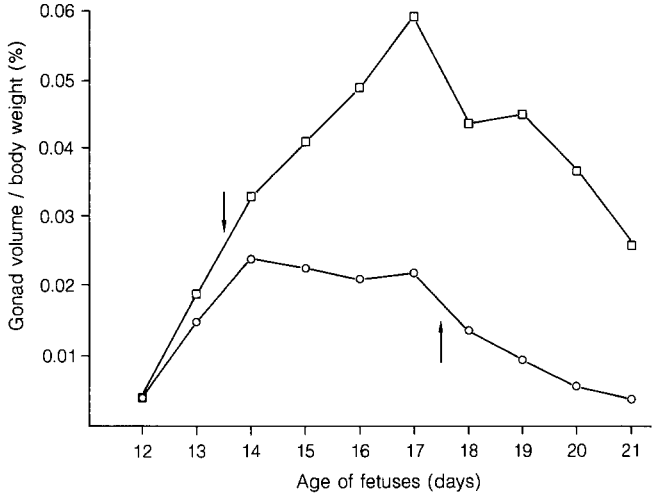

Fig. 4. Growth rates in relation to body weights in XY *(squares)* and XX *(circles)* fetuses of the rat from gonadal ridge formation to birth. *Numbers* on the *vertical axis* refer to mean gonadal volumes in mm^3 per 100 mg body weight. *Upper arrow* indicates the beginning of Sertoli cell formation (Magre and Jost 1980), *lower arrow* the beginning of oocyte formation (Jost 1970). Growth data from Lindh (1961)

based on the extensive data by Lindh (1961) on the rat. At the beginning of gonadal ridge formation on days 12 and 13, the rate of growth of the gonad is very much faster than that of the embryo as a whole in both sexes, with XY gonads growing just a little faster than XX gonads. Between days 14 and 17, accelerated growth continues in the male gonad, whereas the female gonad grows at the same rate a the rest of the body. From day 17 onwards, the gonads grow less fast than the body as a whole in both sexes. Whereas the growth of the XX gonad must be maintained by autosomal and/or X-chromosomal genes, it seems not unlikely that one or more genes on the Y chromosome are involved in the additional growth of the XY gonad.

The first Sertoli cells can be detected in fetuses aged 13 days 9 h (Magre and Jost 1980; Jost and Magre 1988). This takes place initially in the anterior part of the gonad, and extends to the entire gonad during the next 24 h. Meanwhile, a basal membrane forms and encloses the germ cells and surrounding Sertoli cells. Leydig cells are first seen to differentiate by 15 days 13 h.

It is evident that if, as is generally assumed, Sertoli cells take the lead in testicular differentiation, a sufficient number of their precursor cells must be ready to differentiate and to initate the testicular architecture before the germ cells are capable of entering meiosis. Primary occytes in the rat begin to be seen in fetuses of 18 days (Jost 1970). If there are not enough cells capable of becoming Sertoli cells at the beginning of the process, the gonad runs the risk of becoming an ovotestis (Mittwoch 1989). Ensuring the fast development of the gonadal ridge would seem to be the primary task for a Y-chromosomal gene. Now that *SRY* and its homologue in the mouse have been isolated, evidence of their function in the relevant cell types will be awaited with particular interest. In many respects, the differentiation of a testis or ovary from a common rudiment resembles a homoeotic change, as has been previously suggested (Mittwoch 1973).

The Y chromosome and spermatogenesis

Evidence of an association between deleted Y chromosomes and male infertility has increased since Tiepolo and Zuffardi (1976) described 6 men who had azoospermia, and who had *de novo* deletions of the heterochromatic portion of Yq, deletions that may have involved some distal euchromatin. Additional patients with Y-chromosomal deletions have since been reported and provide further evidence that genes located on Yq, particularly in the Yq11 region proximal to it, may be important for testicular maturation and spermatogenesis (Fryns et al. 1985). Two microdeletions in Yq11.23 have recently been reported in men with idiopathic infertility (Vogt et al. 1991b).

In the mouse, it seems that deletions of either the short or the long arm of the Y chromosome can give rise to spermatogenic arrest. The *Sxr* region is derived from the short arm (McLaren et al. 1988; Roberts et al. 1988). A variant of *Sxr*, subsequently named Sxr^b, when associated with a single X chromosome, causes the development of males that are H-Y antigen negative, and whose testes all but lack spermatogenic cells beyond the spermatogonial stage (Burgoyne et al. 1986). This is in contrast to the XO *Sxr* males described by Cattanach et al. (1971), in which all stages of spermatogenic cells were present, sometimes even including a few abnormal spermatozoa. These findings led Burgoyne et al. (1986) to conclude that DNA sequences missing from Sxr^b encode a "spermatogenesis gene", which might be identical with gene for the H-Y antigen. It has been confirmed that Sxr^b arose as a deletion of *Sxr* (Nagamine et al. 1989; Mardon et al. 1989).

Styrna et al. (1991) described a deletion in the mouse giving rise to a Y chromosome that was visibly short in diakinesis, but pairing with the X chromosome seemed unaffected. Males carrying the deleted Y in an inbred strain produced spermatozoa of which 64% had abnormal heads; in F1 hybrids with a number of different inbred strains, the proportion of abnormal sperm varied between 55% and 68%. Some of the sperm had flat acrosomal caps. It is evident that his quantitative effect is not caused by a single gene but could be produced by the transcription of repeated-sequence DNA during spermiogenesis (Burgoyne 1991).

In *Drosophila melanogaster*, the existence of "fertility factors" was proposed when Stern (1929) examined different deletions of the Y chromosome and located a number of regions on both arms, all of which had to be present to ensure the fertilizing ability of the sperm. Later studies revealed the formation, by the Y chromosome, of lampbrush-like loops, which are active in RNA synthesis, in the spermatocytes of larvae (Hess and Meyer 1968). In *Drosophila hydei*, "fertility genes" have been found to consist of repeated units of simple-sequence DNA, the transcripts of which appear as distinct intranuclear structures (Henning 1985). Recently, Vogt et al.

(1991a) have identified a simple-sequence repeat that acts as a "fertility gene" in *Drosophila,* on the human Y chromosome. It will be interesting to see whether untranslatable repeat DNA sequences will turn out to play a role in spermiogenesis.

Conclusion

The two functions carried out by the mammalian testis, the secretion of hormones and the production of spermatoza, develop in sequence; once the first steps of the hormone-producing process have been taken, we are inclined to call the developing gonad a testis and the developing individual a male. However, the production of hormones is, of course, merely an overture to the production of sperm, which will enable the male to reproduce. Since different parts of the Y chromosome are needed to ensure male fertility, it is evident that no single gene can convert the biopotential gonad into a functional testis. It seems that *SRY* functions during the initial stages of testicular differentiation, whereas other Y-chromosomal genes are required to maintain the germ cells and allow their proliferation and differentiation. Even though its effect on the phenotype may be striking, the fitness of the *SRY* gene in the absence of other Y-chromosomal genes is zero. If individuals cannot reproduce, the gender to which they have been assigned is biologically immaterial, and terms like "testis" and "male" are of semantic, social, or legal significance only. Becoming a biologically functional male requires the action of multiple genes on the Y chromosome in addition to *SRY.*

Moreover, many chromosomal anomalies impair or abolish spermatogenesis, even in the presence of an intact Y chromosome. This applies not only to aneuploid conditions, such as 47,XXY and trisomy 21, but also to many apparently balanced translocations, in which spermatogenic arrest may be the only phenotypic abnormality. Theoretically, this could be the result either of a direct effect on spermatogenesis, or of an impairment of the somatic elements of the testis, which therefore provides an unfavourable environment for spermatogenesis. A recent report by Patek et al. (1991) implicates functionally defective Leydig cells in the breakdown of XY germ cells in male XX↔XY chimaeras. As long as the function of the genes on the Y chromosome remains unknown, an arbitrary distinction between "testis-determining" and "spermatogenesis" genes is not helpful in elucidating this problem. Futhermore, testosterone secretion also seems to be increasingly impaired with increasing numbers of X chromosomes. All in all, the idea of the "dominance" of the Y chromosome has outlasted its usefulness. The term "codomiance" would be preferable in taking account of the interaction of Y-chromosomal genes with those present on other chromosomes.

Although the reasons for the adverse effects of chromosomal anomalies on the testis remain to be elucidated, a slowing down of the developmental rate its a likely result of many of them. Now that the need for the fast development of the testis has been demonstrated, it is imperative to identify the agents responsible for the differential growth of organ rudiments. It could be significant that Y-chromosomal effects have been implicated in such variables as testis size in mice (Hayward and Shire 1974; Hunt and Mittwoch 1987) and tooth size in humans (Alvesalo and de la Chapelle 1981). The fact that *SRY* is present in both sexes in a variety of non-mammalian vertebrates (Tiersch et al. 1991) indicates that it originally functioned in both males and females.

It may also be relevant that a growth-regulatory function has been proposed for serologically detectable H-Y antigen (Heslop et al. 1989). This topic appears to be ripe for re-investigation.

Further progress in our understanding of sex determination and differentiation will undoubtedly ensue from different scientific approaches, including studies of metatherian mammals and non-mammalian vertebrates, so that the eutherian condition can be put into a proper perspective. It is to be expected that a better understanding of the subject will give rise to an improved terminology. In particular, the term "sex reversal" is likely to become obsolete, once the relationship between phenotype and the underlying genotype has been properly elucidated.

Spermatogenesis is an extremely complex process, and preparing for it may be particularly arduous for eutherian males, who undergo their early development in a female hormonal environment. The timely interaction of multiple genes leading to the early differentiation of a competent testis appears to be one of the requirements for success.

The evidence shows that the dogma of a single testis-determining gene lacks biological validity, since the development of a fertile testis, and, hence, a male capable of begetting offspring, requires multiple genes on the Y chromosome in conjunction with a single X chromosome. The usually concurrent presence of these units in an appropriate genetic background simulates the effect of a single gene.

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