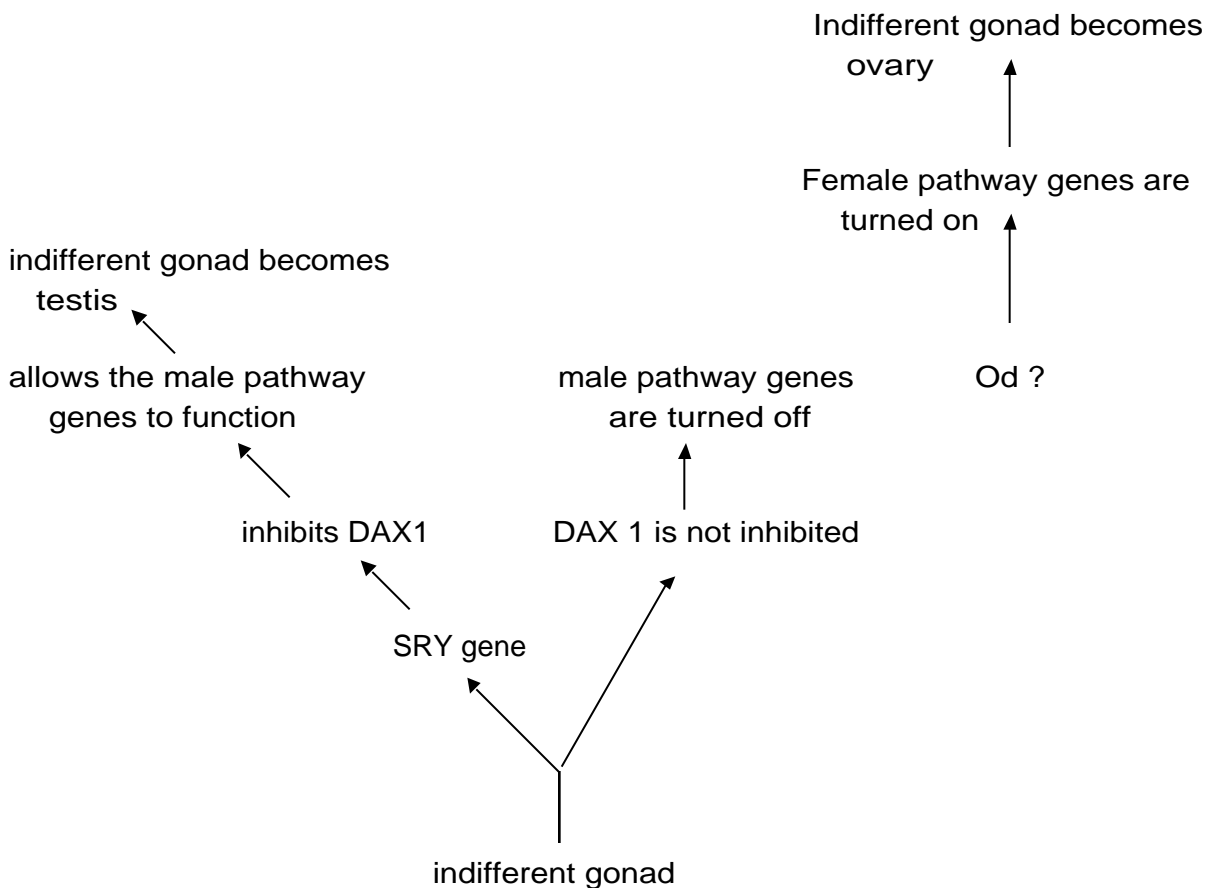


SEX DETERMINATION IN MAMMALS

According to current theory, sex determination in placental mammals is controlled by the sequential action of a series of genes. The normal male mammal has a XY, 2AA karyotype, while the normal female has an XX, 2AA karyotype. ["AA" is used here to emphasize that 2 complete sets of autosomes are also present. This would not be done in a correctly written karyotype.] The Y chromosome contains a gene (SRY) which initiates the male developmental pathway. [The following model for primary sex determination is taken from (1) Eicher & Washburn, 1986. "Genetic control of primary sex determination in mice." Annual Review of Genetics 20: 327-360, (2) Schafer and Goodfellow, 1996. "Sex determination in humans." BioEssays 18(12):955-963, and (3) Jimenez and Burgos, 1998. "Mammalian sex determination: joining the pieces of the genetic puzzle." BioEssays 20(9):696-699.]



Primary sex determination in mammals.

(1) In the mammalian embryo, the indifferent gonad is present as the precursor of the testis or ovary. This indifferent gonad has not yet become committed to either the testicular or the ovarian pathway.

(2) The first gene in the ovarian-determination pathway, hereafter designated ovary determining (Od), is located on either the X chromosome or an autosome. Od initiates ovarian determination by activating the next gene in the ovarian-determination pathway, hereafter called ovary-determining-1 (Od-1). This genetic pathway causes the indifferent gonad to become committed to development as an ovary.

(3) The first gene in the testis-determining pathway, designated sex determining region Y SRY (=Tdy = TDF = Tdf), is located on the Y chromosome. SRY initiates the testis-determination process by inhibiting the expression of DAX1, (which inhibits the male pathway genes from functioning), thus allowing the epithelial cells of the bipotential gonadal primordium to differentiate into Sertoli cells, and thus triggering testis development.

(4) The SRY gene functions in development before the Od gene, thus assuring testicular determination in an XY individual. The gene product of an early male pathway gene may further guarantee testicular determination in an XY individual by inactivating the Od or Od-1 locus.

Secondary Sex Determination.

	<u>Male</u>	<u>Female</u>
<u>Gonad</u>	testis	ovary
<u>Reproductive ducts</u>	The Wolffian duct system develops into the vas deferens, seminal vesicles, prostate gland, etc.	The Mullerian duct system develops into the uterus, oviducts (= Fallopian ducts, Fallopian tubes), innermost portion of the vagina.
<u>Genitalia</u>	penis and scrotum	clitoris and labia and outermost portion of the vagina.

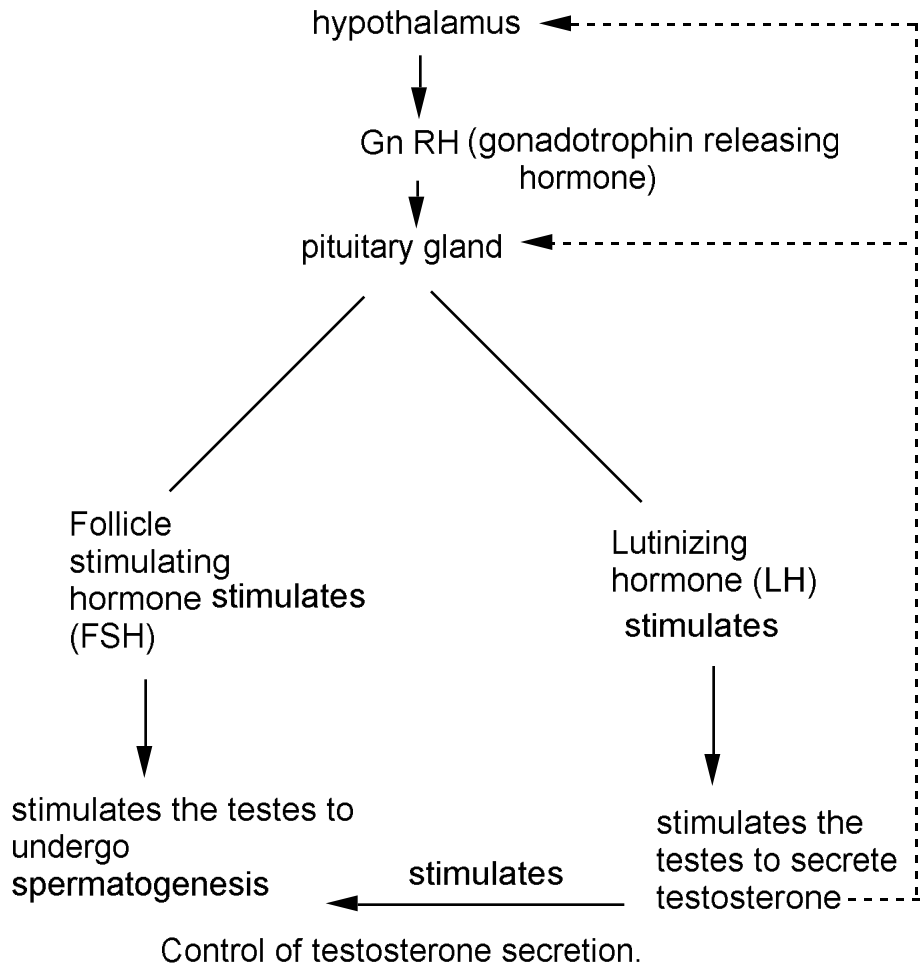
Once the gonads have begun to differentiate, they eventually begin to secrete their appropriate sex hormones. The testes secrete (1) testosterone, and (2) Chi factor (= Mullerian duct inhibitory hormone = AMH, P450 aromatase). Testosterone is secreted by the interstitial cells which are located in the connective tissue between the seminiferous tubules. Testosterone stimulates the testes to develop further, and the seminiferous tubules of the testes to produce sperm. At the same time as the testes are developing under the influence of testosterone, the male duct system (Wolffian ducts) develop into the vas deferens, seminal vesicles, prostate gland, etc. The penis

and scrotal sacs (which are not part of the Wolffian ducts) also develop under testosterone stimulation.

Testosterone also stimulates the development of male secondary characteristics, such as increased growth rate in adolescence, increased deposition of Ca PO₄ in bones, larger bone mass, and greater muscle mass. The deepening of the voice (due to an enlarged larynx), and development of body hair, are also due to testosterone stimulation. The secretion of testosterone is controlled by a negative feedback mechanism. Chi-factor inhibits the development of the female (Mullerian) duct system, which then degenerates and disappears. There is evidence indicating that the chi factor gene may be activated by an early male pathway gene.

In the absence of the SRY gene product, DAX1 continues to inhibit the male pathway and the indifferent gonad develops into an ovary, and secretes estrogens and progesterones. These hormones stimulate the development of the Mullerian duct system of the female into the oviducts (= uterine tubes or Fallopian tubes), uterus, and the innermost portion of the vagina. The indifferent external genitalia develop into the labia and clitoris. Since the testosterone levels are extremely low, the Wolffian duct system fails to develop. Estrogen and progesterone stimulate the development of the female secondary sex characteristics, such as the development of the glandular system of the breasts, deposition of fat on the breasts and hips, etc. Other genes and their products have also been identified in the sex determination process. The exact details of their interactions is still being determined.

The levels of testosterone in the adult male are controlled by a negative feedback system. The hypothalamus (a portion of the brain above the back of the roof of your mouth) secretes GnRH (gonadotrophin releasing hormone), which is carried by a specialized set of blood vessels to the pituitary gland which is located just below the hypothalamus. The GnRH stimulates the FSH and LH centers of the pituitary, and they secrete FSH and LH. The FSH (follicle stimulating hormone) stimulates spermatogenesis in the testes. LH stimulates the testes to produce testosterone. Testosterone stimulates spermatogenesis. High levels of testosterone also inhibit the hypothalamus so that it secretes less GnRH and to a lesser extent, high levels of testosterone also inhibits the hypothalamus so that the levels of FSH and LH drop. This combined inhibitory effect reduces the levels of FSH and LH, and the levels of testosterone also drop. When testosterone levels are low, the testosterone does not inhibit the hypothalamus and pituitary, and FSH and LH levels rise, causing a rise in testosterone levels as well. In short, testosterone levels control testosterone levels. In the adult woman, FSH and LH stimulate the secretion of estrogen and progesterone. High levels of estrogen inhibit the hypothalamus and pituitary, resulting in reduced levels of estrogen and progesterone. This mechanism is further complicated by the details of the menstrual cycle.



Selected genetic abnormalities in human sex determination.

The addition or removal of a single chromosome around the 2n number is referred to as aneuploidy. The presence of one extra chromosome is trisomy, while the presence of one chromosome instead of two is monosomy. Aneuploidies of the sex chromosomes cause abnormal development. An excellent recent reference upon which I relied heavily for my update (and even copied verbatim) is A. de la Chapelle, Sex chromosome abnormalities, in Emery, Alan. E. H., and David L. Rimoin, Ed. (1996). Principles and Practice of Medical Genetics., Churchill Livingstone, N.Y., N.Y..

Klinefelter Syndrome - (47, XXY) The Klinefelter male is sterile. The presence of an extra X chromosome causes a genetic and developmental imbalance which results in a sterile male. The basal membranes of the seminiferous tubules of his testes gradually become replaced with cartilage. Testosterone levels start out low and become even lower. These low levels cause a low sexual libido (sex drive). The penis may be of normal size, or slightly reduced in size. The testes are very small (1-2 cm as compared with 3.5-4.5 cm in the normal male). Many Klinefelter men have some combination of scoliosis (bent vertebral column), enlarged breasts, a female distribution of fat, scanty body hair or a female distribution of sexual hair, and a high pitched voice. The arms and

legs are slightly longer than normal, and he may be 2-5 cm above average height. An abnormal psychological condition occurs in which the man has very little perseverance, he soon ceases to try very hard; whether at his job, or his marriage. He is prone to disturbances of behavior, deviations in personality, and neurotic and psychotic reactions. Alcoholism and other antisocial behavior, including aggressiveness, depression and periods of mania, are reportedly common. Yet in the typical patient these disturbances are seldom severe enough to lead to conviction or admission to a hospital. Rather, the patient tends to lead a quiet, passive life on a low key. His brain waves are also abnormal. The typical patient is of dull normal intelligence or mildly mentally retarded. (He usually has a male sexual orientation, and many patients lead a normal married life.) The extra X chromosome is of maternal origin in 60% of the cases. Extreme Klinefelter males with 48, XXXY or 49, XXXXY are progressively more abnormal, and severely mentally retarded. There are also 46, XY/47, XXY chromosomal mosaics for Klinefelter syndrome, in which the symptoms are variable and usually less severe. In rare instances, mosaics may be partially fertile. The frequency is approximately one in 1000 newborn boys.

Turner Syndrome - (45, X; previously 45,XO). The classical description of Turner syndrome is a sterile female. Her ovaries fail to develop and are found as a streak of connective tissue, referred to as streak ovaries. Primordial follicles are present during prenatal life and at birth, but these degenerate and are gone by the time of puberty. Since the ovaries fail to develop, they do not secrete estrogens or progesterones. The only female sex hormones which occur are produced (in relatively low amounts) by the adrenal cortex. Therefore the secondary sex characteristics fail to develop. The oviducts, uterus and vagina remain infantile, and she does not menstruate. Pubic hair does not grow. The breasts are flat, with the nipples wide-spaced (shield breast). [Actually, there is wide natural variation in the spontaneous occurrence of pubic hair and the development of the external genitalia and breasts in the absence of hormone treatment. Virtually normal secondary sexual characteristics and menstruation can be achieved by cyclic estrogen treatment, although breast development is often reduced.] She often has a "webbed neck" which is a fold of connective tissue on the side of the neck. Her growth rate is reduced, with an average height of 4'8". There is no growth spurt in adolescence. This may be at least partially corrected by prepubertal treatment with growth hormone. Her IQ is not affected, but her personality may fail to mature properly. Descriptions include the terms "goodness, immaturity, childish behavior, non-aggressiveness, overconformity, concrete thinking, lack of originality, childish interests and moralizing attitudes". The missing sex chromosome was often the paternal one, but the reason for the loss is not understood. Turner syndrome occurs with a frequency of one in 2500-3500 newborn girls. Most Turner embryos spontaneously abort. (The syndrome may also occur in XX individuals in which one X is structurally abnormal.)

Triple-X Syndrome - (47, XXX). The triple-X female has variable fertility, ranging from normal to sterile. She may be phenotypically normal, but is often slightly taller (a few

centimeters taller at 172 cm = 5'8") than average (longer legs). According to one source, widely-spaced nipples and webbed neck may be found. The first triple-X women were found in fertility clinics, leading to the assumption that all triple-X women had fertility problems. However, later unbiased studies have shown that many triple-X women have had no trouble having children, and were not detected. According to early studies, menstruation usually begins at an older age, menstrual cycles are often irregular or temporarily interrupted, and menopause begins earlier in XXX women. This has been challenged by more recent studies, and the final answer remains to be seen. Yet most lead a normal sexual life and have children. The extra X chromosome is not usually transmitted to the children. The IQ is usually normal or low normal. In some studies, it was lower (by 5-29 IQ points) than that of their sibs, yet only a few had an IQ lower than 70. Language in XXX children is usually delayed. Learning difficulties are common. Delayed motor development results in awkwardness and more coordination. Emotional maturation may also be delayed. Behavioral problems, including mild depression, conduct disorder, immature behaviour, socializing problems are often found, especially if extra nurturing was not provided. Yet these delays in development can be largely prevented by providing extra increased psychological, social and motoric stimulation at home as well as in school. Tutoring is often needed at some time in their development. The frequency is approximately one in 1000 newborn girls.

XYY Syndrome - (47, XYY). The XYY male was previously reported to be sterile. However, in most XYY men, gonadal development, testicular size and fertility are normal. Testicular histology, including spermatogenesis, has been repeatedly reported as normal and many XYY males are fathers. However, in many instances, smaller-than-normal testes, decreased spermatogenesis, spermatogenic arrest, subfertility and sterility have occurred. The offspring are almost always chromosomally normal, although I have read a few medical reports about the production of XXY and XYY children by XYY men. He is often, but not always, taller than average (some over 2m). His testosterone levels are higher than normal. There is an excess of criminality among XYY males compared to XY males. The kind of crimes are in no way peculiar, resembling those committed by XY men. Thus the postulated preponderance of seriously aggressive behavior against other people - murder, manslaughter, rape, etc. - has not been substantiated. Being XYY does **not** predispose the man to spontaneous acts of violence as was previously thought. Theft, arson, and burglary are the crimes most often cited. The question of what causes the tendency to antisocial behavior which leads to conflict with the law is still not entirely resolved. Impaired intellectual function, poor socioeconomic status, tallness have been suggested as contributing factors, but they are not enough to explain the increased risk of antisocial behavior. The presence of basic genetic defects is indicated by abnormal EEG findings which point to organic defects, i.e., anatomical, histological and/or biochemical abnormalities. Psychological studies have shown that certain personality traits, such as infantilism, lack of emotional control, increased impulsiveness after emotional stimulation and a weak concept of self, are so characteristic that XYY men can be recognized by psychological tests alone. Yet in another study, XYY boys were as a group very little different from

the controls. Early claims that such men are predisposed to acts of spontaneous violence, due to their extra Y chromosome, have thus been proven false by more recent studies. Someone said "he is not aggressive, just impulsive." His IQ is usually reduced (verbal ability in particular). The frequency is approximately one in 1000 newborn boys.

Testicular feminization "Xtfm" - (46, XY). The person who suffers from testicular feminization is a genetic male. The gene which codes for the testosterone receptor molecule is abnormal or absent entirely, and a functional testosterone receptor is not produced. Under these conditions, the Y chromosome containing the SRY gene initiates testicular development by the indifferent gonad. However, the development of the testis is abnormal because it can not be stimulated by testosterone. As a result the testis may develop into an ovotestis, with areas of ovary-like development in the testis. Since the rest of the cells of the body cannot be stimulated by testosterone either, they do not develop in the male pathway. The hypothalamus cannot be inhibited by testosterone, and secretion of GnRH increases. GnRH stimulates the secretion of high levels of LH and FSH by the pituitary. The LH and FSH stimulate the testes to secrete extremely high levels of testosterone (which cannot stimulate the cells due to the absence of a functional testosterone receptor molecule), and lower levels of estrogens and progesterones. In testicular feminization, the levels of estrogens and progesterones approximate those of a normal female. [The normal testes secrete low levels of female sex hormones, and the normal ovary secretes low levels of male sex hormones.] The cells of the male body do make receptor molecules for estrogen and progesterone. Therefore, in this case, most of the body is stimulated to develop in the female pathway. The external phenotype is female, with well developed genitalia (clitoris, labia) and breasts. Pubic hair is typically absent or scant. Internally, the testes migrate to the lower abdomen or labial folds. (They would normally migrate into the scrotal sacs.) The Wolffian duct system fails to develop because it cannot respond to testosterone stimulation. The Mullerian duct system is inhibited by Chi factor, and degenerates. Therefore there are no uterus or oviducts, and the vagina may be shorter than normal. The sexual orientation is female, there is no characteristic psychological abnormality, and she may have a normal marriage. She is, of course, sterile. [There are other genetic forms of this syndrome in which there may be less female-like development. These forms usually involve a low level of testosterone binding by receptor molecules.]

THE BALANCE CONCEPT OF SEX DETERMINATION IN DROSOPHILA.

From a genetic view point, the fruit fly, *Drosophila melanogaster*, is one of the best studied organisms in the world. It was soon discovered that normal males were (8, XY) and normal females (8, XX). Early workers had also identified abnormal (7, XO) sterile males and (9, XXY) fertile females. Bridges published the results of crosses between unusual triploid females (12, XXX) and normal males. The resulting offspring varied in chromosome number and sexual phenotype. An analysis of his results demonstrated that most of the female-determining genes are on the X-chromosome

and autosome IV (which is very small). Most of the male-determining genes are located on autosomes II and III. (The X-chromosome is #1 in this species.) The Y-chromosome is not male-determining in *Drosophila*, however it does contain genes controlling spermatogenesis.

Triploid females are fertile and can be identified by their thick heavyset bodies, coarse bristles and coarse faceted eyes. One such female (Bridges 1921) produced 9 normal males, 96 females, and 37 exceptionally large individuals which showed various body malformations (in the eyes, bristles and wings) and proved to be totally infertile. They were especially remarkable for their poorly developed gonads and ducts. The external genitalia often appeared a mixture of rudimentary male and female parts, and in some of these flies one ovary and one testis were present together. Sex combs presented various degrees of malformation, color and shape of the abdomen appeared as a patchwork of male black and female pale color.

In this series of intersexes, which showed an extremely variable mixture of male and female characteristics, nearly male and nearly female specimens were present but they could always be distinguished from normal male and female individuals because of their resemblance to triploid individuals. They could thus be divided into male type and female type intersexes, and cytological and genetic analysis demonstrated that the male type possessed three #II, three #III chromosomes, two #IV and two X chromosomes. The female type of intersexes possessed three #II, three #III, three #IV, and two X-chromosomes, (or else four sets of autosomes and three X-chromosomes). The presence or absence of a Y-chromosome exerted no influence on sex expression in any class of intersexes. (Metafemales and metamales are poorly viable, late emerging and sterile. Their phenotypes are essentially normal female or male.)

Ratio of X Chromosomes to Autosomes and Corresponding Sex Type in *Drosophila melanogaster*.

X chromosomes (X) and sets of autosomes (A)	Ratio X/A	Sexual Phenotype
♀ 3X 2A	1.5	metafemale (sterile, low viability)
4X 3A	1.33	metafemale (sterile, low viability)
2X 2A	1.0	*female (fertile)
3X 4A	0.75	intersex female type (sterile)
2X 3A	0.67	intersex female type (sterile)
2X 3(-IV)A	0.67	intersex male type (sterile)
1X 2A	0.5	*male (fertile, if Y present)
♂ 1X 3A	0.33	metamale (sterile, low viability)

The regular decrease in the X/A ratio which is observed in the series going from the metafemales to the metamales through the series of intersexes and standard female and male phenotypes, has shown a relationship between the X and the

autosomal sex determining genes which forms the basis of the theory of quantitative genic sex balance. The increasing proportion of autosomes in the various chromosome complements shown in the table corresponds to an increase of male characteristics in the different sex phenotypes and these observations confirm the previous conclusion both autosomes, and X chromosomes play an important role in the determination of sex. Thus the evidence supports the concept of female determining genes on the X-chromosome and male determining genes on the autosomes. However, the general description must be modified somewhat. Chromosome IV is weakly female determining (and shows some tendency to pair with the X). [The genes on the IV of *D. melanogaster* are homologous to X-linked genes in other *Drosophila* species.]

"HAPLO-DIPLO" AND POLYGENIC SEX DETERMINATION IN HYMENOPTERA.

In Hymenoptera, wasps, bees, ants, etc., the original system of sex determination is a "**haplo/diplo**" (haploid/diploid) system, in which females are diploid and males are haploid. Female progeny are produced from fertilized eggs, while male progeny are produced from unfertilized eggs. Female insects have a supply of sperm cells from previous matings which they store in a "sack" called the spermathecum. In the wasp *Habrobracon juglandis* (= *Bracon hebetor*), when an egg passes down the oviduct of mated females sperm may or may not be released from the spermathecum. If they are, the egg is fertilized and produces a female. If they are not, a male is produced instead. [In a lab strain of this species the usual sex ratio is 67% females to 33% males.] Superimposed on top of this haplo-diplo system is a system of **sex alleles**. The normal diploid female is heterozygous for 2 different sex alleles (s_1s_2, s_3s_4 , etc.), while the normal male (which is haploid) is hemizygous for one sex allele (s_1, s_2, s_3, s_4 , etc.). Individuals which are homozygous for one sex allele (s_1s_1, s_3s_3 , etc.) are either dead or sterile males, depending upon the species.

SEX DETERMINATION IN BIRDS.

Birds have heterogametic ZW females and homogametic ZZ males. The W-chromosome is small and mostly heterochromatic like the mammalian Y-chromosome. Sex is apparently controlled by a Z/A balance system similar to the X/A system of *Drosophila*, except that the male-determining genes are on the Z chromosomes, and the female-determining genes are on the autosomes. The W chromosome is not female determining in birds!

SEX DETERMINATION IN LEPIDOPTERA.

ZZ-ZW sex determination is also found in Lepidoptera, some fish and some amphibians. In the few species of Lepidoptera which have been studied, the female determining genes are on the W chromosome and the male determining genes are on the Z chromosome. The autosomes are not sex determining.

SEX DETERMINATION STUDY QUESTIONS.

1. Describe and discuss sex determination in *Drosophila melanogaster* in detail. (Include the intersexes and their significance in your presentation.)
2. List the total number of chromosomes, the sex chromosome makeup, and the phenotypic characteristics for Klinefelter syndrome.
3. List the total number of chromosomes, the sex chromosome makeup, and the phenotypic characteristics for Turner syndrome.
4. List the total number of chromosomes and the phenotypic characteristics for XYY syndrome. Include the questions of criminal and aggressive behavior.
5. List the total number of chromosomes and the phenotypic characteristics for XXX syndrome.
6. Describe and discuss the role of hormones and hormone receptor molecules in sex determination. Give examples supporting your statements.
7. Describe the sex determination system found in birds.
8. Describe the sex determination system found in Lepidoptera (moths and butterflies).
9. Describe the sex determination system found in Hymenoptera (wasps, etc.).
10. Describe the adult phenotype of testicular feminization in detail. Describe and discuss how and why the development of a person with this syndrome differs from the development of a normal male. Include the mutant gene, its effects on gonads, hormones, reproductive ducts, external genitalia and psychological development.
11. Write the sexual phenotype (such as normal male, metafemale, etc., including fertile or sterile) for each of the following *Drosophila melanogaster* karyotypes:
 - a) XXY, (2)II, (2) III, (2) IV
 - b) XXX, (2)II, (2) III, (2) IV
 - c) XX, (3)II, (3)III, (2)IV
 - d) X, (2)II, (2) III, (2) IV
 - e) XY, (3)II, (3)III, (3)IV
 - f) XX, (2)II, (2) III, (2) IV
 - g) X, (3)II, (3) III, (3) IV
 - h) XX, (3)II, (3)III, (3)IV
 - i) XY, (2)II, (2) III, (2) IV