chapter III

Sex Determination: An Overview
Reproduction, variation and inheritance of variations are the three basic features of any living system. Genetics is the science of genes and their inheritance, and DNA the genetic material, is inherently organized to undergo reproduction, variation and inheritance of variation in cellular organisms. The basic sources of such variations are mutation and recombination that cause biological evolution. Sex implies being male and female and sexual reproduction involves mating of male and female for production of genetically variable progenies that result from gametic recombination of parental genomes during sexual reproduction. Genetics of sexuality therefore comprises genes of maleness and femaleness as well as genes that cause and promote gametic recombination. There are variety of sexual systems functioning in microbes, plants and animals with the sole common purpose of promoting genetic recombination and producing genetically different recombinant progenies.

Genetic knowledge of sex and sexual reproduction is known to have played indispensable role in the past in generating breeds of animals and plants with improved products for our ever-growing need of survival and civilization. Sexual reproduction is also a natural means to repair and maintain the structural and functional integrity of genome. It is therefore, not surprising to know that genetics of sex-determination and evolution of sex chromosomes have attracted best minds of the world to understand their evolutionary causes and consequences.

Studies on genetics of sex-determination in various model animal systems have shown an astonishing variety of genetic and epigenetic sex-determining systems that operate to achieve the same purpose of producing two sexual types, the male and female. Now it is clear that a common principle operates behind the observed variations in genetic mechanism of sex-determination. This common principle consists of a primary sexual signal that can be genetic or environmental and is different in males and females, and a key sex-specific regulatory gene, whose state of activity is transmitted through a cascade of subordinate regulatory genes to the sex-differentiation genes, determining maleness or femaleness. Does such a common principle imply that the genes and the molecular mechanisms of sex determination in various animals have been conserved in evolution? The available information suggests that the
nature of the genes and the associated molecular mechanisms have not been conserved during the course of evolution (Schütt and Nothiger, 2000). This fact would become self evident during the brief description of genes involved in determining and controlling sexual phenotypes, in *D.melanogaster*, *M. domestica*, *C. elegans* and humans.

3.1 Sex Determination in Lepidoptera

Diptera (flies) and lepidoptera (moths and butterflies) are the insects of monophyletic group but the former has XY system of male heterogamety and the later has a ZW system of female heterogamety. However, variations in sex chromosome system are known to occur with XX female and XO male in diptera and ZZ male and ZO female in lepidoptera. Partial or total heterochromatinization is another feature of lepidoptera sex chromosomes (Traut and Marec, 1997). In the somatic cells of lepidoptera, W-chromosome forms a conspicuous heterochromatic mass. Due to its derivation from W and its female specific presence the heterochromatin is designated as "W chromatin" or sex-chromatin (Traut, 1999).

*B. mori* females are designated as ZW and males as ZZ. Females of other lepidopteran species may be ZO, ZW, ZZW or ZZWW, the last being the case of presumed fragmentation of W (Traut and Mosbacher, 1968; Soumalainen, 1969; Robinson, 1971). Hashimoto (1933, cited in Tazima, 1964)) was the first to suggest that the W-chromosome has a female determining role in the silkworm on the basis of the fact that silkworms with ZW, ZZW and ZZWW were all females (cited in Tazima, 1964). Tazima confirmed this model of sex determination in *Bombyx* and showed that sex determinants are localized at one end of the W chromosome (Tazima, 1964; Strunnikov, 1983). Changing in the ratio of sex chromosome to autosome had no effect on sex-determination and gynandromorphs (i.e., sexual mosaics), instead of intersexes, were produced. This suggested that there is a balance of active factor on both W and Z-chromosomes (Tazima, 1964; Robinson, 1971).
Unlike males, lepidopteran females have no crossing over. W and Z-chromosomes are paired in the pachytene stage. Chromosomes with all the signs of non-homology do synapse along their entire length. However, complete alignment of homologous chromosomes by synaptonemal complex in pachytene does not lead to crossing over and chiasmata formation. The absence of crossing over has led to the evolution of a substitute for chiasma at diplotene. The substitute consists of modified synaptonemal complexes that are not eliminated at the end of pachytene as in the case in chiasmatic meiosis (Traut and Marec, 1996).

Deficiencies of the Z-chromosome are lethal in females whereas deficiencies of one of the Z-chromosomes do not have any effect on the sex expression in males (Tazima, 1964). The presence of a piece of W-chromosome, not including the female determinant (transferred to chromosome-3 by reciprocal translocation) has a lethal effect in males while the loss of this portion of W chromosome has no effect on sex expression in females. These findings indicate that the Z and W chromosomes in B.mori have at least some non-homologous segment containing sexually antagonistic genes (Tazima, 1964; Rasmussen, 1978; Traut and Marec, 1996).

The lepidopteran sex heterochromatin (W-chromatin) is transcriptionally inactive. This has been demonstrated by its failure to label in the presence of tritiated uridine in the silkglands of Ephestia (Traut and Scholz, 1978). Nevertheless, uridine incorporation is associated with heterochromatin in nurse cells indicating that at least part of the W-chromosome remains active in tissues involved in sex-determination (Guelin, cited in Traut and Scholz, 1978). These kinds of observations have led to the suggestion that heterochromatinization serves to reduce or eliminate the function of the W-chromosome in selected tissues, analogous to the role of chromosome diminution in Ascaris, Cyclops and Sciara (Traut and Scholz, 1978; Crouse, 1990).
3.2 Sex Determination in *Drosophila melanogaster*

Expression of *Sxl* in somatic cells is regulated by a cell autonomous signal, the X:A ratio but its expression in XX germ line requires both the cell autonomous signal of X:A ratio and an inductive signal generated by somatic cells (Bakerc and Beloti, 1983; Steinmann-Zwicky et al., 1989). On the contrary, in *C. elegans*, the same set of genes that control the somatic sex also control germ line sex but with some basic difference in their mode of action in the two cell lines (Schedl and Kimble, 1987, 1988; Villeneure and Meyer, 1990).

*SXL* protein controls *Sxl* and *tra* transcript splicing directly by binding to poly U-rich stretches in pre-mRNA (Sosnoski et al. 1989; Inove et al. 1990; Valcarcel et al. 1993). The gene *snf*, *vir* (*virilizer*), and *fl(2)d* [*female-lethal (2)d*] generate products that facilitate SXL’s positive autoregulatory splicing (Granadino et al., 1990; Flickinger and Salz, 1994; Hilfiker et al., 1995). The switch gene *tra*, the direct regulatory target of *Sxl* requires a partner *tra-2* (*transformer-2*) (Fujihara et al., 1978). Together these two genes control the sex-specific alternative RNA splicing of *dsx* (*double sex*), an unusual bifunctional gene (Nagoshi et al., 1988; Mattow et al., 1990). A likely target for *tra* in the sex determination pathway is *fru* (*fruitless*) (Gailey and Hall, 1989; Gailey et al. 1999).

*Dsx* (*doublesex*), is the gene controlling terminal differentiation of somatic or germ cells into male/female phenotype. It acts downstream of *tra* and *tra-2* (Baker and Ridge, 1980). DSX protein works both by repressing gene expression that is appropriate for differentiation of opposite sex, and by inducing gene expression appropriate to the same sex (Coschigano and Wensink, 1993; Jursnich and Burtis, 1993). It is assisted by partners (genes) which are not sex specifically regulated: *ix* (*intersex*) and *her* (*hermaphrodite*) (Cline and Meyer, 1996).

The *Sxl* gene responds to the dosage of cis elements under the influence of X:A ratio in early *embryogenesis* and becomes set in one of the two modes,
either active (females) or inactive (males). The early phase of control also involves the activity of other genes such as da, liz, fl(2)d. The active state of SXL protein in turn acts on the tra gene transcript to promote its correct splicing for functional TRA protein. The combination of active TRA protein and TRA-2 protein, a distinct product of tra-2 gene together function in correct splicing of dsx gene transcript to produce $\text{DSX}^F$ protein which causes development of female phenotype by activating female pathway genes and suppressing the transcription of male specific genes. The whole sequence of events thus described is characteristic of female sexual phenotype development in XX individuals. In males, in the absence of either TRA or TRA-2, male specific default splicing results in $\text{DSX}^M$ proteins (Cline and Meyer, 1996).

In contrast, the product of Sxl gene in XY individuals, where X:A ratio is 0.5 remains inactive. Since active state of Sxl is required for production of active TRA protein, the tra gene product naturally remains inactive. Under such situation dsx gene transcript undergoes default mode of splicing leading to the production of $\text{DSX}^M$ protein that inhibits expression of female specific genes and promotes expression of male specific genes thus causing the XY individuals to become males (Cline and Meyer, 1996) (Fig. 3.1).

### 3.3 Sex Determination in *Musca domestica*

*M. domestica* is a dipteran member like Drosophila and exhibits male heterogametic type of sex determination system. However, it differs from Drosophila in the following basic respects: It has a transposon like male determiner gene 'M', epistatic to female determiner gene 'F,' and known to function as determiner of male phenotype by inactivating the female determining gene 'P'. In this system, absence of 'M' in homogametic XX system ($\text{F}+/\text{F}+$) results in induction of female pathway (Schmidt et al., 1997a; Schmidt et al., 1997b). This is in sharp contrast to Drosophila system in which the master sex specific switch Sxl controls not only sex-specific pathways but also dosage compensation pathways. Such functional involvement of Sxl in Drosophila suggests that its sex-determining mechanism has become refractive
Fig. 3.1. The somatic cascade for sex determination in *Drosophila melanogaster* (Adapted from Hodgkin, 1990)
to further evolutionary changes. This is not the case in *M. domestica* as it lacks dosage compensation and its key sex-specific genes seem to function exclusively in sex- determination (Dübendorfer *et al.*, 1992; Schutt and Nothiger, 2000). It is worthwhile to mention here that *M. domestica* contains homologue of Drosophila *Sxl*, whose expression is not controlled by the primary sex-determining signal of X:A ratio therefore not equivalent to ‘F’ gene, whose expression is regulated by primary sex determining signal ‘M’. Similar results have been obtained in other non-Drosophilid species. In conclusion, it thus seems that original function of *Sxl* gene was not specific to sex-determination in non-Drosophilids and that its role as a coordinator of sex-determination and dosage compensation was acquired during evolution of sex in Drosophila (Meise *et al.*, 1998; Schutt and Nothiger, 2000).

### 3.4 Sex Determination in *Caenorhabditis elegans*

The level of HER-1, the product of the key regulatory gene *her-1*, determines the activity of other sex-determining genes lying downstream of *her-1*. The sequential interaction of sex-determining genes in *C. elegans* unlike that in *D. melanogaster* is negative (Hodgkin, 1987). In other words, *her-1* gene product is inhibitor of *tra-2*, *tra-3* gene products, which in turn inhibit the activity of *fem 1,2,3* genes. Such sequential interactions result in high level of *tra-1* gene product in XX individuals thus causing them to develop into female. In XO individuals, the similar negative interaction results in low level of *tra-1* gene product leading to male soma. In fact, the state of *dsx* gene product regulates maleness and femaleness in Drosophila and the level of *tra-1* gene product regulates maleness/ femaleness in *C. elegans* (Hodgkin, 1986; Cline and Meyer, 1996) (Fig.3.2).

### 3.5 Sex Determination in Mammals

The observation of heteromorphic sex chromosomes in humans, an XX pair in females and an XY pair in males, suggested a chromosomal basis of sex determination but was not informative as to the mechanism. The description of
Fig. 3.2. The Somatic and Germline cascade inferred for *C. elegans*.
(Adapted from Hodgkin, 1990)
human phenotypic female with a single X-chromosome (Ford et al., 1959) and a phenotypic male carrying two X-chromosomes and a Y-chromosome (Jacobs and Strong, 1959) demonstrated that, rather than X:A ratio acting as a signal for sex determination, the presence of Y-chromosome directs male phenotypic development and in its absence, a female sexual phenotype results (Schafer and Good fellow, 1996).

Discovery of a mutant mouse with chromosomal complement of a female (XX), but developing as a male, designated as Sxr (sex reversed) by Cattanach et al., (1971), was an important step in understanding the mechanism of sex determination. Singh and Jones (1982), using the highly conserved Bkm probe showed for the first time that sex reversal in these mice was due to translocation of a very small part of the Y-chromosome to the distal end of the long arm of the X-chromosome. Bkm studies revealed that the Sxr region of the mouse Y-chromosome is strongly male determining and is necessary and sufficient to convert a chromosomally female mouse into a male (Singh and Jones, 1982). A systematic analysis of XX males and XY females has led to the detailed mapping of Y-chromosome (Guellaen et al., 1984; Disteche et al., 1986; Page et al., 1987) and isolation of ZFY (Zinc finger on the Y-chromosome), earlier thought to be testis-determining factor (TDF) on the Y (Page et al., 1987) and SRY (Sinclair et al., 1990), the gene in the sex reversal region of the Y which has now been shown to be the regulatory switch for sex-determination. SRY is required for triggering the differentiation of bipotent gonad primordium into 'sertoli' cells leading to formation of testis. Once the testis is formed, the male hormones they produce give rise to the male phenotype. In addition to SRY, several other genes like DAX1, SF1, WT1, DMRT1 are suggested to be involved in determination of male and female sexual phenotypes in mammals (Jimenez and Burgos, 1998; Raymond et al., 1999). The activities of SF1 and WT1 are essentially required for the development of bipotential gonads from genital ridge (Kreidberg et al., 1993; Luo et al., 1994; Graves 1998).
In addition, SF1 gene is also expressed in sertoli cells during gonad formation, which implies an important role of this gene in testis development (Ikeda et al., 1994). Further studies on the role of this gene in testis development have shown its essentiality for the activation of anti-mullerian gene (AMH gene) and steroidogenic gene (Shen et al., 1994). DAX1 has been shown to act as the inhibitor of SF1 gene function leading to inhibition of testis development (Swain et al., 1998).

SRY gene product is proposed to induce testis development indirectly by antagonizing the suppressive effect of DAX1 gene on SF1 gene product (Jimenez and Burgos, 1998). Consequently, such interaction between SRY and DAX1 gene product results in normal activity of SF1 gene product, which in turn activates the male pathway of testis development. The testis thus developed produce male hormone for determining male phenotype as well as anti-mullerian hormone that inhibits the pathway of ovarian development (Jimenez and Burgos, 1998).

Recently, a gene with DM-domain similar to mab-3 and dsx has been identified in humans and designated as DMRT1 (Raymond et al., 1998). DMRT1 maps to the small region on 9p24.3 implicated in sex reversal and is a strong candidate gene for this trait (Raymond et al., 1999). DMRT1 is expressed exclusively in the genital ridge before sex-differentiation (the only other gene which shows this pattern is SRY,) and soon after is expressed only in the testis (Raymond et al., 1999), thus DMRT1 seems to be involved in mammalian sexual development (Ellegren, 2000).

According to the current status of sex determination in mammals, a model has been proposed to account for the genetic basis of maleness in XY individuals and femaleness in XX individuals (Fig.3.3). However, the fact that a large number of individuals having normal SRY and other genes implicated in sex-determination, with no mutation at all, develop as females and a large number of individuals without the presence of SRY develop as males and none of the hermaphrodites contain any 'Y' DNA at all suggests the involvement of other genes, which have not yet been identified (Thangraj et al., 1998).
Fig. 3.3. DSS gene based model for sex determination in mammals. (Adapted from Jimnez and Burgos, 1998).
3.6 Dosage Compensation

The evolution of heteromorphic chromosomes will in most cases lead to a significant imbalance in the dosage of the genes located on the sex chromosomes which may have nothing to do with sex determination but are nevertheless present in two doses in one sex and single dose in the other. Some form of dosage compensation is likely to occur in order to equalize the activity of all these genes between the two sexes (Hodgkin, 1992). The experimental analysis shows that haploidy or triploidy for more than a few percentage of the total genome is severely deleterious or lethal so evidently compensation will be an essential process. This experimental finding has led to the concept that dosage compensation may not occur for those sex linked genes which are very few in number or are required to express only in one sex or the genes which are part of gene families distributed throughout the genome. Accordingly, lack of dosage compensation for sex-linked genes in birds and butterflies has been explained on the basis of very small size of sex chromosomes carrying very few genes (Johnson and Turner, 1979; Baverstock et al., 1992; Hodgkin 1992).

However, the lack of dosage compensation in various systems reported so far still requires scientific explanation as to why dosage compensation is not required for the viability of ZZ/ZW sexual system but required for the viability of the XX/XY system.

3.6.1 Dosage Compensation in Lepidoptera

In female heterogametic organisms like birds, butterflies it has been confirmed that the sex linked genes are not dosage compensated (Cook, 1964; Johnson and Turner, 1979; Barestock et al., 1982; Suzuki et al., 1997). In Heliconius melpomere and H. elato, the activity of 6-phosphogluconate dehydrogenase (6-PGD) located on the Z-chromosome is not compensated between the two sexes (Johnson and Turner, 1978). As in the case with other lepidoptera, B. mori has female heterogamety and does not show dosage compensation. The
level of transcription of Z-linked genes shows a two-fold difference between males and females. As for example *Bm kettin* gene, homologue of the Drosophila *Kettin* gene, is located on the Z-chromosome and is not dosage compensated, as its transcript level is two fold higher in males than in females. Unlike the dosage compensated species such as mammals in which one copy of the homogametic chromosome pair (XX) is inactivated in the somatic tissues by heterochromatinization (Lyon, 1974), in many lepidopteran species as well as other taxa with heterogametic females like birds and snakes there is ample evidence that it is W-chromosome that becomes inactivated (Cock, 1964).

Lack of dosage compensation supports the idea that female limited characters will be predominantly autosomal whereas male limited sexual characters can be both sex linked and autosomal (Johnson and Turner, 1979). Among butterflies, a large proportion of genes controlling female mate selection are located on the Z-chromosome (Grula and Tayler, 1980) and the genetic data on these phenotypes suggest that most, if not all, of the Z-chromosome lacks dosage compensation (Johnson and Turner, 1979; Grula and Taylor, 1980). Thus, it appears that there is a close relationship between an absence of dosage compensation of Z-linked genes and sexual dimorphism in phenotype.

### 3.6.2. Dosage compensation in *Drosophila melanogaster*

*Drosophila* has impressive list of molecules that associate with dosage compensated X-chromosome of XY individuals thus causing two-fold increase in the transcription rate of X-linked genes. They include male X-chromosome proteins like MLE, MLS-1, MLS-2, MLS-3, MOF and non-coding RNAs like *roX-1* and *roX-2* (Gorman *et al.*, 1995). The male X-chromosome protein and *roX-1* and *roX-2* are required together for binding to the target sites located on the male X-chromosome where they catalyze acetylation of histone H4 at its 16-lysine residue. This interaction opens the chromatin for two-fold rise in transcription of X-linked genes (Baker *et al.*, 1994). In males the dosage compensation system works mainly because SXL protein in male cells is rendered inactive thereby not interfering with the activity of *msl-2* gene product,
MSL-2 protein (Bashaw and Baker, 1995; Kelley et al., 1995). In comparison, in females SXL protein remains active leading to inhibition of the activity of MSL-2 protein. Since MSL-2 protein in association with other protein components and roX-1 and roX-2 RNA, is a basic requirement for two-fold rise in transcription from X-linked genes (Birchler, 1996), its inactivity in female cells naturally results in no influence on basic rate of transcription of X-linked female genes. This is how equalization of the level of X-linked gene product occurs in XX and XY cells of fruit fly (Cline and Meyer, 1996; Stuckenholz et al., 1999).

3.6.3. Dosage compensation in *Caenorhabditis elegans*

In nematodes, the dosage compensation mechanism acts to down regulate transcription of X-linked genes to 50% in XX individuals and not in XO individuals (Hodgkin, 1983). The reason is that SDC-2 and SDC-3 proteins are XX individual specific and not XO individual specific. Since, the complex down regulating the transcription of X-linked genes is made of protein subunits like SDC-2, SDC-3, DPY-26, DPY-27, DPY-28, MIX-1 and since SDC-2 and SDC-3 are made only in XX individuals and not in XO individuals, naturally the down regulation of X-linked genes occurs specifically in XX individuals and not in XO individuals (Lucchesi, 1998).

In XX individuals, (high X:A ratio) expression of *Xol-1* gene is reduced. Since the product of *Xol-1* gene inhibits the formation of *Sdc-1* and *Sdc-2* genes product, the level of SDC-1 and SDC-2 protein naturally remains high in XX individuals. High level of these two gene products in turn reduces the product of *her-1* gene and causes the formation of active protein complex called DPY complex down regulating these expression of X-linked genes by 50%. The sequence of events found occurring in XX individuals is reversed in XO individuals where *her-1* gene product level is raised and as a result the production of active DPY complex fails to occur. Thus, DPY complex system is the mechanism of dosage compensation in *C.elegans* (Cline and Meyer, 1996; Lucchesi, 1998).
3.6.4. Dosage compensation in mammals

In mammals, dosage compensation involves inactivation of all X-chromosomes except one regardless of sex chromosome constitution (XO, XY, XX, XXY, XXX etc). Obviously, dosage compensation mechanism in mammals must be capable of sensing number of X-chromosome present for inactivation. The mechanism of X-inactivation is known to result from the activity of $Xist$ gene whose product is a 15kb long non coding and cis acting signal originating from the Xic centre (Kelly 1995; Penny et al., 1996; Backdroff et al., 1998). The $Xist$ RNA produced from the inactive X-chromosome coats its source. The Xic locus is a multifunctional locus involved in cis propagation of X-chromosome inactive state through $Xist$ RNA production, as well as in the decision of how many and which chromosome to be inactivated. The molecular details of such inactivation process are far from clear (Brown et al., 1991; Backdroff ef al., 1992; Rastan, 1994).

3.7. Evolution of Sex Chromosome

The existence of specialized sex determining chromosomes poses a range of fascinating and fundamental questions, few examples of which have received any satisfactory answers. Why, for example, has the evolutionary process led to such developments? Why do some, but not all species exhibit them? How it is that an entire chromosome, originally containing many other genes unconnected with sex, can become subverted to a singular developmental programme? Why does the functional monosomy, involved in the specialization of the Y or W sex-determining chromosome, not lead to chromosomal imbalance and consequent lethality? Related to this, why is there compensation for X-linked gene dosage in mammals, but dosage effect in respect of Z-linked genes in reptiles? Could it be that dosage compensation is not the primary function of X-inactivation? We do not know the answers to many of these questions. However, attempts have been made to answer some of these questions.
Chromosomal sex determination and sex chromosome evolution are the two dissimilar distinct biological processes linked evolutionarily. It is an observed fact that Y or W chromosome (or part of them) forms a genomic compartment that does not recombine with the respective X or Z-chromosome. Suppression of recombination between X/Y and Z/W results in differentiation of the two chromosomes Y and W by separate evolutionary mechanisms of degenerative nature. Taxonomic distribution of heteromorphic sex chromosomes suggests that sex chromosome heteromorphism must have evolved independently on many occasions. This indicates that conditions predisposing the specialization of sex chromosomes are wide spread amongst eukaryotes and the probability of their evolving is relatively high. Most often it has been assumed that the heterochromatin, which is a common feature of sex-determining chromosomes throughout vertebrates, is the end point of a long and complex process of mutational drift to functionlessness associated with gradual 'compensation' for hemizygosity (Ohno, 1967; Charlesworth, 1978).

The Z and W-chromosomes of birds share many features with mammalian X and Y-chromosomes respectively. Both avian chromosomes are metacentric, pair during meiosis and a synaptonemal complex is formed at the ends of the short arm of the two chromosomes, therefore a small pseudoautosomal region exists (Stefos and Arrighi, 1971; Ellegren, 2000). W-chromosome forms a sex-chromatin (W-chromatin) in female snake somatic tissues. It remains in the condensed state during meiosis and conformationally it is out of synchrony with the Z-chromosome during pachytene (Ray-Chaudhari et al., 1971; Ray-Chaudhari and Singh, 1972). Similar behavior of W-chromosome has been shown in moth *Ephestia kuchniella* (Traut and Scholz, 1978). Such a relatively discrete condensation cycle obviously would be consistent with the activity of one, or few, genes and its timing is appropriate for early embryonic development including, presumably, sex determination. Inactivated mammalian X-chromosome becomes reactivated in oocytes (Gartler et al., 1972; Lyon, 1974).
It has generally been accepted that sex was determined initially by an allelic difference at a locus born on a homologous pair of \textit{autosomes}. The two primordial sexes consisted of individuals heterozygous or \textit{homozygous} at the sex-determining locus. The transformation of the autosomes bearing the sex-determining gene in question into heteromorphic sex chromosomes is thought to have resulted from preferential accumulation of loss of function mutations in the neighborhood of the sex-limited allele of the gene. The retention of such mutations was facilitated by a general reduction in the rate of recombination in the genome of individuals bearing the sex-limited allele or atleast in the chromosomal region adjacent to the allele (Lucchesi, 1999). A number of authors such as Brian, Charlesworth, James Bull and others have proposed models that would account for a reduction in recombination between the \textit{homologues} bearing the sex-determining genes. These models are all derived from R.A. Fisher's idea that, if mutations with opposite effect on the sexes occur on the chromosomes bearing the sex-determining locus, a tighter linkage of these mutations to the sex-determining gene will be selected in order to maintain the appropriate favorable allele in the appropriate sex (Bull, 1983). Sex-limited mutations benefiting one sex may accumulate if they harm the other sex due to counter selection (Lucchesi, 1999).

\textbf{Heterochromatin} preceded gross structural alteration of the W in evolution. Structural alterations of the W-chromosome have played a minor causal role, if any, in its evolution and it also implies that the molecular alterations involved in becoming \textit{heterochromatic} can occur relatively rapidly in evolutionary time. This further implies that functional \textit{monosomy}, which accompanies this process of chromosomal inactivation, can also be accommodated without a prolonged evolutionary adjustment or 'compensation' for altered gene dosage. The fact that there is no evidence of dosage compensation in reptiles, birds or lepidoptera (Ohno, 1967; Johnson and Turner, 1979; Baverstock et al., 1982) further suggests that there was little or no barrier to prevent a rapid evolution of the W-chromosome (Jones, 1983).
In other words, acquisition of sex-determining function and suppression of recombination are prerequisites for chromosome pair to undergo differentiation by Muller’s Ratchet (Muller, 1964, Charlesworth, 1978), by sex specific acquisition of \textit{Bkm} sequences (Singh, 1976) or by genetic hitchhiking (Rice, 1987). Why does such suppression of recombination between \textit{heteromorphic} sex chromosome pair occur at all? Bull (1983) suggested that most likely reason is that if there are sex linked alleles with opposite effect on fitness in the two sexes, there is a selective advantage in reducing recombination between the sex determining locus and these genes. According to Rice (1987), suppression of recombination is selectively favored by the accumulation of sexually antagonistic genes on the sex chromosome.

Meiotic cell division is a known mechanism for chromosomal recombination to occur in eukaryotic cells. The function of meiotic recombination has been found to be the means for producing genetically variable progenies. In addition, it is also known to play indispensable role in the repair of both genetic and epigenetic defects in DNA and in the elimination of selfish DNA like transposons from the 	extit{germline} (Bernstein, 1977, Martein, 1977, Holliday, 1984, Ettinger, 1986]. Accordingly, recombination deficiency can result in the accumulation of mutation and other genetic changes in the \textit{heteromorphic} chromosomes. The significance of suppression of crossing over between the sex chromosomes in the \textit{heterogametic} cells was first pointed out by Muller (1914, 1918). He suggested that lack of crossing over in XY heterogametic males would cause accumulation of deleterious recessive mutations on Y-chromosome leading gradually to its progressive \textit{heteromorphism}, a phenomenon termed as Muller’s Ratchet. There is an alternative view that finds experimental reasons to conclude a specific role of \textit{Bkm} sequences in heteromorphic degenerative evolution of Y or W sex- chromosome and where the entire chromosome seems to function like a super gene (Singh, 1995) According to Jones (1983) \textit{W-evolution} is triggered by mutations, which cause the control of the process of mitotic W-condensation to be modulated by the sex-determinants. According to this model, the cycle of somatic inactivation and germ cell activation of the W-chromosome signifies the cycle of sex gene
expression. In species where $W$ has continued to specialize, the sex determiner assumed control over the center, involved in chromosome condensation. A spreading effect or read through from the sex gene was such that when it turned off, the chromosomes also condensed; the sex determiner thus fortuitously but effectively had hijacked the entire chromosome. In primitive species, the model postulates that the condensation center was not located sufficiently close to the sex determiner for there to be a similar interaction between them. In evolutionary biology, sexual selection based on sexual heteromorphism seems to have played a very important role in the evolution of various systems for regulating the genetic interest of the two parents. Few examples of such systems include dosage compensation, genomic imprinting and haplodiploidy system (Moore and Haig, 1991).

Meiotic pairing is normally dependent on structural and conformational homology between the two chromosomes involved. In respect of sex chromosome this can be illustrated in terms of behaviour of sex chromosomes in somatic and reproductive cells. In heterogametic XX/XY system, the XX females exhibit heterochromatinization of one of its $X$-chromosome in somatic cells and its euchromatinization in oocytes. In contrast, XY males exhibit euchromatinization of its $X$ in somatic cells and heterochromatinization in spermatocytes. The problem of inadequate pairing in non-homologous region is avoided when these regions either become heterochromatinized or euchromatinized. These ideas have led Jablonka and Lamb (1990) to suggest that the need to avoid effects of pairing failure may have played an important role in the evolution of sex-chromosome heteromorphism. According to them, reduced crossing over between the sex-chromosomes, functional and structural degeneration of the $Y$-chromosome, sex-chromosome imprinting and dosage compensation may all be evolutionary consequences of the requirement for pairing at meiosis. There are two pathways, conformational and structural, known to be associated with initiation of reduced recombination in the non-homologous regions of the heteromorphic sex chromosomes. Conformational pathway assumes that the event initiating evolutionary changes leading to sex-chromosome heteromorphism was a change in chromatin conformation in the
region in which the sex determining genes were located. The structural pathway assumes that the initiating event was a structural change resulting from an inversion or a translocation in the originally homomorphic sex chromosomes (Jablonka and Lamb, 1990).

### 3.7.1 Conformational Pathway

There are many examples in both vertebrates and invertebrates where the only known difference between the sex chromosomes is a conformational difference. The genus *Triturus* has XY system of sex determination and the two sex chromosomes in somatic cell can be recognized only by specific staining for constitutive heterochromatin, which is found greater in amount on Y chromosome (Schmidt et al., 1979). Similarly, in the Cyprinodont fish, *Poecilia sphenops* v. *melanistica* with WZ system, the W-chromosome of the female differs from Z in having a large terminal heterochromatic segment (Haaf and Schmid, 1984). Singh et al., (1976) showed that W-chromosome to differs from Z-chromosome in having a specific satellite DNA in evolutionarily intermediate group of primitive snakes containing otherwise seemingly homomorphic sex chromosomes.

The conformational difference between chromosomal regions reflects differences in the level of condensation of chromatin (Schweizer et al., 1987), the timing of replication in 'S' phase (Holmquist, 1987) and level of sensitivity to Dnase I (Kerem et al., 1983, 1984). Such chromatin organization confers on it a conformationally active or inactive state. Reduced recombination in conformationally heteromorphic region of XY chromosomes in species of *Triturus* has been experimentally demonstrated (Schmid, 1983), thus confirming the fact that conformational heteromorphism between the regions of homologous sex chromosomes is the cause of suppression of crossing-over. Ray-Chaudhari et al., (1971) suggested another mode of chromatin conformational heteromorphism resulting from late replication of DNA in the W-chromosome of WZ snake system. According to them, late replication of DNA in 'W' is the first step in the differentiation of 'W' from the 'Z' chromosome in snakes (Ray-Chaudhari and Singh, 1972). Schempp and Schmid (1981), have
made similar observation with European frog, *Rana esculenta*. Singh and his group have shown involvement of sex specific *Bkm* sequences (GATA sequences) in controlling Y or W-chromosome heteromorphism associated with their male or female specific gene activity. According to this model, *Bkm* sequences would also result in suppression of recombination between non-homologous regions of the sex chromosome (Jones, 1983; Jones and Singh, 1984).

### 3.7.2 Structural Pathway

There is considerable evidence suggesting that in many different groups the evolution of sex chromosome heteromorphism has involved a structural change such as pericentric or paracentric inversion or a translocation (Ohno, 1967). The structural change, which usually involves only one of the two homologues, can have two rather different effects, which can contribute to the functional degeneration of ‘Y’ or ‘W’ chromosome. First, a small inversion or a translocation can result in incomplete pairing either within the rearrangements or in adjacent region (White, 1973). To counteract the adverse effects of pairing failure during meiotic prophase, any mechanism that inactivates the unpaired region will be selected. In this way both X and Y-chromosomes could come to have genetically inactive regions.

Structural change may also contribute to the functional degeneration of Y chromosome through its effect on recombination. Crossing over between structurally heterozygous chromosomes frequently results in production of inviable gametes carrying chromosomal duplications or deletions. Evidently, many structural changes act as apparent crossover suppressors thus causing progressive degeneration of Y-chromosome.

Lepidoptera contains *WZO/ZZO* system of sex-determination in which W is suggested to have arisen from free homologue of the fused *autosome* to Z chromosome (Traut and Marec, 1997). Another example is *Drosophila miranda*. This species has a neo Y-chromosome formed by a fusion between the ancestral Y and an autosome. Steinemenn (1993) has shown that the
originally **autosomal** part of the neo Y has some regions, which are heterochromatic and have accumulated repetitive DNA sequences which are not present on the original homologue thereby implying a role of repetitive DNA in degeneration of Y-chromosome.

### 3.8 Sex Chromosome Evolution In Lepidoptera

Marked differences are observed between 'advanced' families that form the clade ditrysia comprising 98% of all lepidopteran species and the primitive non-ditrysian families in respect of sex chromatin. All ditrysian families include species with sex chromatin \((WZ/ZZ)\) system and sporadically species without sex chromatin \((Z/ZZ)\) system. In contrast none of the species investigated from non-ditrysian families had sex chromatin \((Z/ZZ)\) system. The inference is that the W-chromosome had not evolved when these non-ditrysian lineages diverged from the common lepidopteran stem (Traut and Marec, 1997; Traut, 1996).

A possible scenario for the acquisition of the W is fusion of an autosome with the Z-chromosome. The free homologue of the fused autosome is then transmitted as a W-chromosome in female lineage. All molecular and morphology differentiation of the WZ pair must have taken place since then including the loss of the W-chromosome in some ditrysian species. Lepidoptera thus, displays the full evolutionary life cycle of a univalent sex chromosome, through genetical and structural differentiation, rearrangement with **autosomes** and eventual loss (Traut and Marec, 1997; Traut, 1999).

### 3.9 Junk DNA and Sex-Determination

Early work on organization of eukaryotic genome by using renaturation kinetics (Britten and Kohn, 1968) revealed that a large fraction of the genome consists of non-coding repetitive DNA sequences, also called as Junk DNA. It has been estimated that 97% of the human DNA is junk or **non-genic** and the remaining 3% is **genic** (Nowak, 1994). However, recent studies on this aspect are clearly providing evidence for indispensable role of Junk DNA in the regulation and
organization of centromere, telomere, chromosomal architecture and gene activity. The following gives a brief account of the known families of the junk DNA (repeated DNA sequences) characteristic of eukaryotic systems with their possible known functions.

1. **Introns**: Most eukarotic genes contain introns that do not code for proteins. Some of the introns are now known to code for small nucleolar RNA (sn RNA) believed to be essential for the assembly of ribosomes (Elder and Turner, 1995).

2. **Satellites**: They are short DNA sequences 140-180bp long, repeated 100-1000 times at a stretch. They mainly occur at the ends and centers of the chromosome. They are essential for the structural and functional integrity of chromosomes as their absence leads to chromosomal disintegration.

3. **Minisatellites**: Similar to satellites but are shorter up to 40bp long, occur throughout the genome. Defective minisatellites are found to be associated with cancer disease.

4. **Microsatellites**: They are shorter than minisatellites, usually 2-5bp long. GATA sequences of Bkm are examples of microsatellite and are known to be essential for the organization and activity of heteromorphic sex chromosome (Singh, 1995). In addition, GATA motif and GATA family of transcription factors are known to be essential for the activity of globin and other erythroid specific genes (Evans et al., 1988; Mignotte et al., 1989).

5. **SINES** (non-LTR) and **LINES** (LTR): They occur in numerous copies. One example of SINE is 300bp Alu sequences that occur 500,000 times in human genome. LINES are similar to SINES but longer up to 700bp apiece. Both LINES and SINES hop about the genome and cause mutation if they land in a gene (Nowak, 1994; Elder and Turner, 1995).
Haploid genome of silkworm, *B. mori* contains 560 million bp/silk gland cell which is approximately 3.5 times the size of *D. melanogaster* and 1/6th to the size of human genome (Nagaraju, 2000). Molecular characterization of repetitive DNA elements have shown the occurrence of microsatellites of dinucleotide repeats (CA)_n and (CT)_n, transposon like elements characteristic of Drosophila genome and retroposons typical of mammalian genome. In addition, it also contains SINES like Bm1 and Bm2 representing 5-10% of its total genome. The additional DNA sequences present in silkworm genome are diverse types and include Pao, Mag, R1Bm, R2Bm2, BmC1, RDM, R2DM, Jockey, Fgi, and mariner (Nagaraju, 2000).

With the advent of recombinant DNA technology, it became quite feasible to probe the occurrence and activity of non-coding regulatory DNA sequences like GATA and coding genic sequences like globin gene, DAX1, SRY, etc. Bkm sequences have been used as a probe to locate its presence on autosomes and sex chromosomes in various animal systems belonging to snakes, birds, mouse, and humans.

The female Banded Krait (*Bungarus fasciatus*) is the source of Bkm DNA sequences (Singh *et al.*, 1980). Further studies showed the presence of cross-hybridizing Bkm sequences in various eukaryotes from slime molds to humans (Singh *et al.*, 1984; Arnemann *et al.*, 1986). Cloned Bkm positive genomic fragments of Drosophila and mouse contain long clusters of tetranucleotide GATA repeats, as a component (Singh *et al.*, 1984). Further studies showed long stretches of GATA repeat alone giving similar hybridization pattern as did the original Bkm probe (Schaffer *et al.*, 1986; Traut 1987).

*Bkm* sequences exhibit a tendency to be concentrated on sex chromosomes thereby implicating their definite role in the sex determining process or in the allocyclic behavior and evolution of sex chromosomes (Jones 1984; Singh *et al.*, 1984). They are predominantly located on the W-chromosomes of snakes (Singh *et al.*, 1980) and on the X-chromosome of *D. melanogaster* (Singh *et al.*, 1981). Similar studies in mice showed the presence of a cluster of Bkm
sequences in the proximal regions of the Y that is essential for the sex
determination. Close linkage of \( Bkm \) to sex determination can be seen in Sxr
mice in which sex reversal is regularly associated with an exchange of \( Bkm \)
cluster to the X-chromosome (Singh and Jones, 1982). However, \( Bkm \)
sequences are known to occur on autosomes as well in \( Ephestia kuehniella \)
(Traut, 1987) and mouse (Kiel-Netzger et al., 1984).