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Chapter 3

SEX DETERMINATION IN INSECTS

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CONTENTS

I. INTRODUCTION

Sex determination is the process by which the gender of a bisexual organism becomes fixed, so that the individual progeny develops either as a son or a daughter. As is the case with other fundamental biological processes, evolution has in the course of time produced a seemingly infinite variety of ways of achieving this one essentially simple objective, and classical genetic and cytogenetic observations have, over the years, combined to display a bewildering diversity of sex-determining mechanisms. Much of this work has been on insects, from the first recognition of sex chromosomes in the heteropteran *Pyrrhocoris apterus*,[1] through the classic experiments of Bridges[2-4] on *Drosophila* and Goldschmidt[5,6] on *Porthetria dispar*, to the recent molecular work elucidating the hierarchy of regulatory genes responsible for the sex of fruit flies.[7,8]

In the general literature on sex determination, two works stand out,[9,10] each with a radically different approach to the subject. Both cover the full range of sex-determining systems, but the cytogeneticist White[9] gives pride of place to evolutionary changes in the sex chromosomes, whereas the evolutionary geneticist Bull[10] pays more attention to the underlying

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mechanisms. The problem with reviewing sex determination in insects at the present point in time is that, for all orders except Diptera, the greater part of the evidence is cytological, and even the most basic information about the genetic systems involved is not usually available. It is nevertheless worthwhile to follow the lead of Nöthiger and Steinmann-Zwicky,[11] and look for general principles of sex determination that could be applicable to all insects, and possibly to all biparental organisms.

In this chapter I shall start by reviewing the main types of sex determination found in insects, then outline what is known about sex chromosome systems in each insect order, and end with some discussion of the evolutionary implications of what we now know about sex determination.

II. GENERAL ASPECTS OF SEX DETERMINATION IN INSECTS

The sex of an insect is almost always determined genetically. Hermaphroditism, in the sense of the same genotype producing functional male and female organs in the same individual, seldom occurs in insects; it seems to have evolved only once, in one genus of scale insects (*Icerya*). An oft-quoted second example of insect hermaphroditism, in the termiticolous phorid group Termitoxeriinae, has now been refuted.[12] (True hermaphrodites should not be confused with gynandromorphs and intersexes — genetically abnormal individuals — which are of common occurrence in insects.) Environmental factors may sometimes influence the genetic determination of sex (see Bergerard[13] for review), but the type of environmental sex determination that occurs widely in reptiles, for example, where the sex of an individual is decided by the environment of the egg after it has been fertilized, seems to be rare in insects.

A. XX/XY SYSTEMS

Most bisexual organisms produce a 1:1 sex ratio, and this can be achieved simply by having one sex (the **heterogametic** sex) produce two genetically different types of gamete, and the other sex (the **homogametic** sex) produce gametes of only one of these types. The two types of gamete carry different **sex factors** (here given the notation s_1 and s_2), which segregate from one another in Mendelian fashion in the meiosis of the heterogametic sex:

$$s_1s_1 \times s_1s_2 \text{ (parents)} \rightarrow 1{:}1\ s_1s_1 \text{ and } s_1s_2 \text{ (progeny)}$$

The heterogametic sex is the male in most insects, but female in Lepidoptera, Trichoptera, and some Diptera.

Although these sex factors are conventionally regarded as alternative alleles at the same locus, it is usually the case that only one sex factor plays an active part in the determination of sex. The other "sex factor" may merely be the corresponding site on a homologous chromosome: e.g., a nonfunctioning (null) allele, or the location at which the sex factor is inserted, in the case of a transposable element.

In some cases, sex factors may be inherited as single genes, recombining freely with other genes on the same chromosome pair, although they are more often than not tightly linked to other genes involved in sex differentiation. Very often, however, chromosomes carrying sex factors are cytologically distinct (heteromorphic), so that the inheritance of sex can be observed cytologically:

$$XX \times XY \text{ (parents)} \rightarrow 1{:}1\ XX \text{ and } XY \text{ (progeny)}$$

X and Y chromosomes usually pair at meiosis before segregating to opposite poles, but there is normally little or no recombinational exchange between them. This is called an XX/XY, or XY (male) sex determination system. When the female is the heterogametic sex, the sex chromosomes are sometimes termed Z and W, and the system called ZW/ZZ:

ZW (female parent) × ZZ (male parent) → 1:1 ZZ and ZW (progeny)

but this terminology has now been largely abandoned in the literature on insect cytogenetics as an unnecessary complication. The fact that the female is the heterogametic sex can be indicated by putting the heterozygous genotype first; i.e., XY/XX or XY (female) sex determination.

At this stage it is important to address two common misconceptions about sex chromosomes, which can easily hinder understanding of sex-determining mechanisms and their evolution. The first point concerns the common notation of heteromorphic sex chromosomes throughout both plant and animal kingdoms as X and Y, which might be thought to imply some degree of homology, not just at the sex-determining loci but of the chromosome as a whole, across major groups of organisms. On the contrary, there is no doubt that heteromorphic sex chromosomes have evolved many times independently in different taxa.[14] The Y chromosome in particular can sometimes be an extremely labile structure, apparently undergoing cycles of degeneration and regeneration within taxa. These will be discussed further later, and in order to emphasize this lability it is sufficient here to note that what seem to be major changes in sex chromosome constitution, such as the formation of a Y chromosome *de novo* (a "neo-Y"), can be found even within a single species.

The second common misconception is that the Y chromosome always has a dominant, male-determining function. This is certainly the case in mammals and in some insects, but the more general condition in insects with XX/XY systems is for the Y chromosome to have an essentially passive role, influencing sex merely by segregating opposite the X at meiosis. The sex of the zygote is then determined by the balance between the actions of regulatory genes on the X chromosome and on the autosomes. This is roughly equivalent to the "genic balance" model developed by Bridges[3] from his work on *Drosophila*. The zygote must be homozygous for a sex factor in order to be female (XX), so this has also been termed a "recessive-X system".[10]

Genetic studies are obviously needed to establish for certain whether the system operating in any one species is based on a dominant Y or genic balance. For species with heteromorphic sex chromosomes, deductions are possible by observing the sex of individuals with abnormal sex chromosome constitutions. The two most informative abnormalities are XXY and XO. If XXY individuals of a species which normally has an XX/XY system are male, then there is obviously a dominant Y factor operating, but if XXY is female, then the Y chromosome is likely to be sexually inert, and the X has an active, although recessive, role. Frequently, aberrant individuals completely lacking a Y chromosome (i.e., with XO constitution) are at least viable enough to observe their sex; such individuals will be female in dominant-Y systems, but male in genic balance systems, as the latter require XX for female determination. Evidence like this is available for only relatively few insects, mostly Diptera, in which occur both dominant-Y (e.g., *Phormia regina, Lucilia cuprina*[15]) and recessive-X systems (e.g., *Drosophila melanogaster*,[3] *Glossina palpalis*[16]). However, there are good reasons for believing that genic balance is the most general condition in insects, stemming from the widespread occurrence of XX/XO sex determination in many insect orders.[9]

B. XX/XO SYSTEMS

In organisms with heteromorphic sex chromosomes, X-Y recombination is usually suppressed, and the Y chromosome tends to be more degenerate than the X, often having few or no functional alleles. This degeneration of the Y is generally perceived as a progressive evolutionary phenomenon.[10] Various explanations for this have been offered.[14,17] For example, because the Y is permanently heterozygous and nonrecombinant, selection must act at the level of the entire chromosome, so it evolves as "an asexual component of an otherwise sexual genome".[18] Deleterious mutations (often nonfunctional alleles) will tend to accumulate in the absence of recombination by the process known as "Muller's ratchet,"[19] which may be

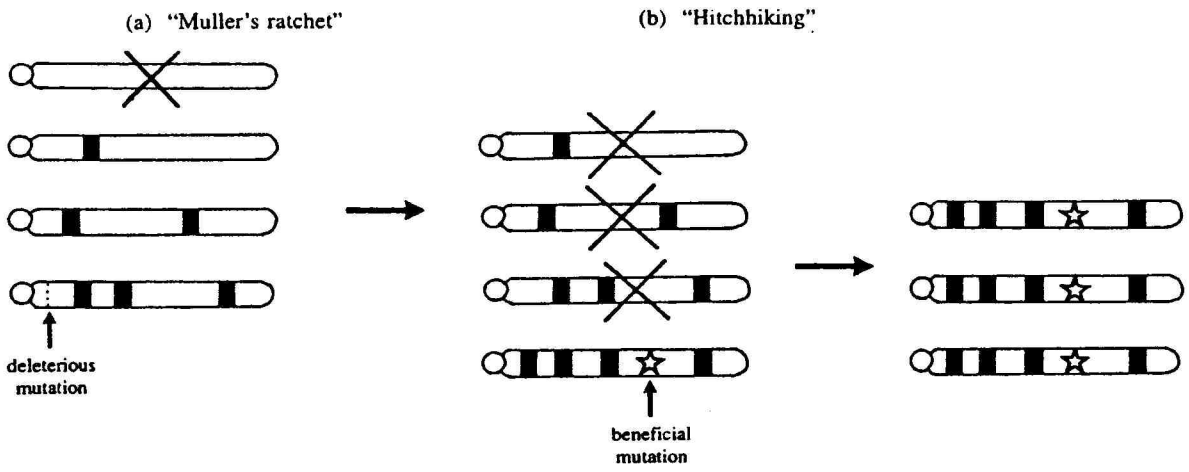

FIGURE 1. Degeneration of a Y chromosome by operation of (a) Muller's "ratchet," and (b) "hitchhiking." In a population of Y chromosomes free from recombination (left), some Ys will have one or more mutations to nonfunctional alleles (black segments), with different Ys mutated at different loci. If the selective disadvantage per locus is small and population size is small enough relative to the mutation rate, the class of Y chromosomes with no mutations may be lost by chance and, because there is no recombination, it cannot be restored. Next the class carrying just one mutation becomes vulnerable to chance loss, and so on, so that as time goes on the mean numbers of mutations per Y chromosome gradually increases. If a favorable Y-linked mutation should occur, however (☆), it could spread rapidly through the population by selection, carrying with it any nonfunctional alleles that happen to be present on the Y chromosome in which it originated ("hitchhiking"), leading to fixation of nonfunctional alleles and accelerating the degenerative process.

accelerated by a "genetic hitchhiking" effect[18] (Figure 1). However, there is still no entirely satisfactory explanation.

The end point of such an evolutionary process may be the complete loss of the Y chromosome, so that males are XO. An XX/XO sex determination system works in exactly the same way as an XX/XY system, with the X moving to one pole in male meiosis, so that sperm are either with or without an X:

$$\text{XX (female parent)} \times \text{XO (male parent)} \rightarrow \text{1:1 XX and XO progeny}$$

Obviously, since there is no Y, such a system must be based on genic balance. XX/XO sex determination occurs in almost all orders of insects, both primitive and advanced. It is almost certainly the ancestral form of sex determination of orthopteroid insects and probably of other major orders such as Hemiptera and Coleoptera. It is even possible that the ancestors of all insects had XO males, and that all insect Y chromosomes have arisen *de novo*.

Y chromosomes in insects often seem to arise as a result of centric fusion between an X chromosome and an autosome (e.g., Figure 2). A recently formed, autosomally derived Y chromosome (a neo-Y) is often easily recognized because it is likely to be still homologous with the autosomal part of the neo-X, and therefore synapses with it at meiosis. This homology may be gradually lost in the course of evolution as X-Y recombination becomes suppressed, and secondary structural and genetic changes occur independently on both the neo-X and the neo-Y.

Clearly, a neo-Y chromosome cannot carry a dominant factor for male determination, and sex determination in such cases must be based on genic balance, as in the ancestral XX/XO system. Neo-XY systems occur most frequently in groups in which XX/XO systems are common; these include the orthopteroid orders, Odonata, the hemipteroid orders, and Coleoptera. Each of these groups will be discussed later. In all of them, there are also species with multiple sex chromosomes.

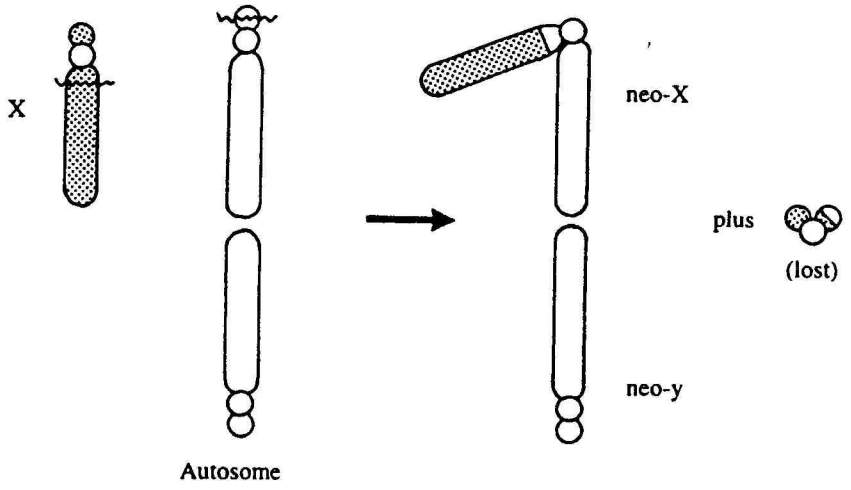

FIGURE 2. White's[9] model of the origin of neo-XY sex determination from an XO condition in the heterogametic sex. After breakage near the centromeres of an X and an autosome, centric fusion occurs, creating a neo-X chromosome. When this fusion has reached fixation in the population (i.e., when the original unfused Xs no longer occur), the original homologue of the autosome involved in the fusion will be confined to the male line and will act as a "neo-Y", segregating opposite the neo-X at meiosis. (White[9] believed that centromeres were never situated at the extreme ends (telomeres) of chromosomes, and therefore his models assume arm breaks and exchanges, followed by loss of a minute chromosome. Alternative models of centric fusion involving breakage *within* centromeres are discussed by John and Hewitt.[19a])

C. MULTIPLE SEX CHROMOSOME SYSTEMS

Chromosomal differences between male and female insects normally involve only one chromosome pair (X and Y, or just the X in XX/XO systems), but there are numerous cases in most insect orders where the difference between the sexes involves a larger number of chromosomes. The most common "multiple" sex chromosome systems have just two Xs (the notation used for the male condition X_1X_2Y, or X_1X_2O, and the notation for the sex determination system is $X_1X_1X_2X_2/X_1X_2Y$, or $X_1X_1X_2X_2/X_1X_2O$), although species are known with almost any number of Xs from 1 to 6 (and in one extreme case, 12), and any number of Ys from 1 to 6.

The origins of $X_1X_1X_2X_2/X_1X_2Y$ sex determination are usually fairly simply explained, with good evidence provided by the way in which the X_1, X_2, and Y associate at metaphase I of spermatogenesis. Figure 2 showed the origin of a neo-XY condition by centric fusion, resulting in a metacentric X (i.e., with the centromere near the center of the chromosome) and an acrocentric Y (with the centromere at one end). If a further centric fusion should occur, this time between the Y chromosome and another acrocentric autosome, then an X_1X_2Y condition will arise, with a metacentric Y (Figure 3).

Alternatively, an X_1X_2Y condition may be derived directly from an XO, by a reciprocal translocation between the X and one member of an autosome pair, the other member of the autosome pair becoming a neo-Y (Figure 4).

The other common derivation of a multiple sex chromosome system is that found in organisms with holocentric chromosomes (i.e., chromosomes with diffuse centromeric activity), such as the Hemiptera and Dermaptera, where X_1, X_2, etc., have almost certainly arisen simply by dissociation (fission) of the single original X chromosome into two or more parts, which still segregate to the same pole at meiosis (Figure 5). Such X dissociations have occurred in both XX/XY and XX/XO systems, a single X dissociation giving $X_1X_1X_2X_2/X_1X_2Y$ and $X_1X_1X_2X_2/X_1X_2O$, respectively. Multiple Ys have also arisen by dissociation in some species. It is characteristic of multiple sex chromosome systems formed by dissociation that the X_1, X_2, etc. are much smaller than the original X, and that there is no accompanying change in the number of autosomes.

Multiple sex chromosomes are cytogenetically interesting, because they show very clearly the fixation of different types of chromosomal rearrangement, but they have little or no

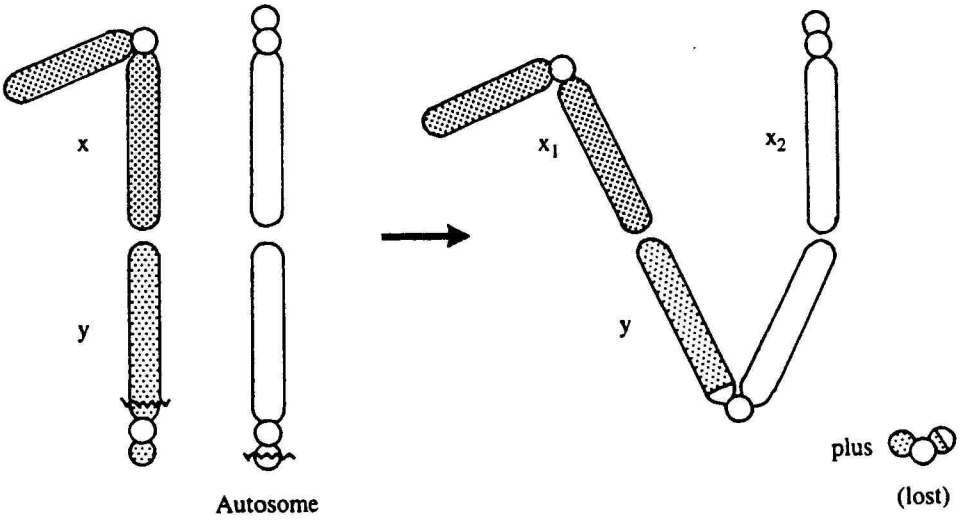

FIGURE 3. Origin of an X_1X_2Y condition in the heterogametic sex (leading at fixation to an $X_1X_1X_2X_2/X_1X_2Y$ sex determination mechanism), by centric fusion between an acrocentric Y and an acrocentric autosome, to give a metacentric "neo-Y" ("neo" because part of it is recently derived from an autosome), and a neo-X_2. The arrangement of sex chromosomes on the spindle of the first meiotic division, with homologous sections associated, is shown diagrammatically. (Adapted from White, M. *Animal Cytology and Evolution*, 3rd ed., Cambridge University Press, Cambridge, U.K., 1973.)

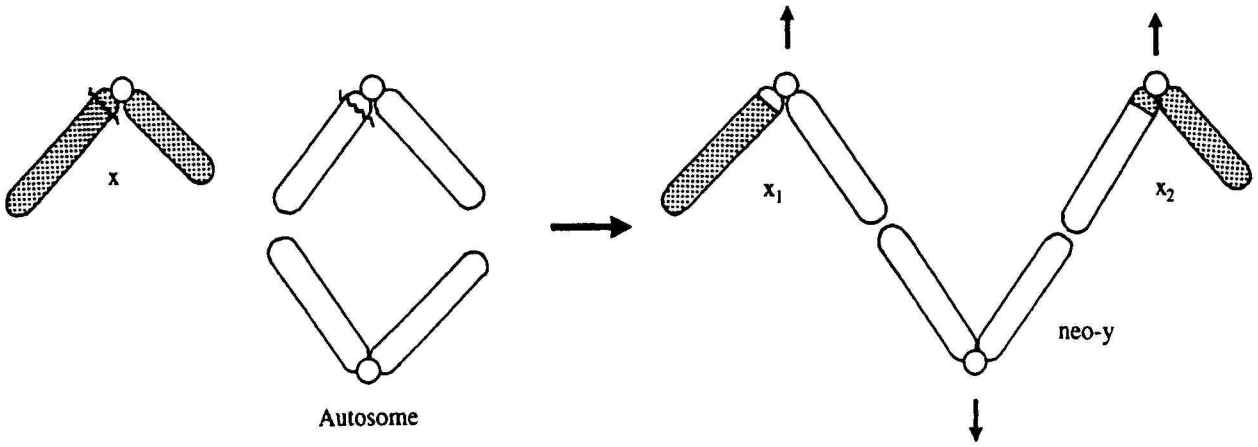

FIGURE 4. Origin of an X_1X_2Y condition in the male (leading at fixation to $X_1X_1X_2X_2/X_1X_2Y$ sex determination) from an XX/XO system, by reciprocal translocation between a single metacentric X in the male and a metacentric autosome. The arrangement on the spindle of the first meiotic division, with homologous sections associated terminally, and X_1 and X_2 moving to the opposite pole from the Y chromosome, is shown diagrammatically. (Adapted from White, M. *Animal Cytology and Evolution*, 3rd ed., Cambridge University Press, Cambridge, U.K., 1973.)

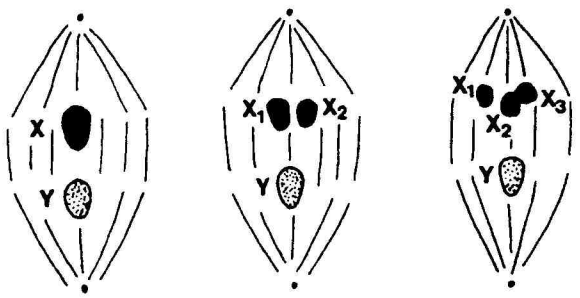

FIGURE 5. Multiple X chromosomes (in [b] and [c]) are derived from an XY system (a) by simple dissociation. Segregation of Xs and Y at spermatogenesis is represented diagrammatically, with autosomes not shown. Usually in such systems the Xs and Y do not associate, but show "distance" (or "touch-and-go") pairing.

significance in relation to the molecular genetic basis of sex determination. Of more interest in this respect are the so-called "multiple factor" systems of certain Diptera.

D. "MULTIPLE FACTOR" SYSTEMS

Species with multiple sex chromosomes are nevertheless still likely to have only a single sex-determining locus — for example, on just one of the Xs in a multiple X system. But more complex sex-determination systems are known where one species may have sex-determining factors at several different loci, and sometimes even different individuals within the same population have their sex factor on different chromosomes. Such complex systems cannot be detected cytogenetically, and they have only been recognized in genetically well-studied organisms with an abundance of genetic markers. In insects, this means certain species of Diptera.

The type of multiple factor system most commonly observed in Diptera and found in members of both the Nematocera and the Cyclorrhapha, can be symbolized in a general way as follows

Females	**Males**
$s_1s_1\ s_2s_2$	$S_1s_1\ s_2s_2$
	$s_1s_1\ S_2s_2$

S_1 and S_2 behave as dominant male-determining factors, segregating, respectively, at the two different loci 1 and 2, and always restricted to the male line. This 2-locus system, with S_1 and S_2, can be generalized to any number of loci:

Females	**Males**
$s_1s_1\ s_2s_2\ s_3s_3....$	$S_1s_1\ s_2s_2\ s_3s_3....$
	$s_1s_1\ S_2s_2\ s_3s_3....$
	$s_1s_1\ s_2s_2\ S_3s_3....$
	⋮

So for any species with a multiple factor system, there is always just one female genotype, homozygous for all sex factor loci, and *n* distinct male genotypes, each heterozygous at just one of the *n* loci. Sometimes a sex factor locus may be on a cytologically distinct sex chromosome, so that S_1, for example, is manifestly a Y chromosome; but sex factors may equally occur on chromosomes that are in all other respects autosomal, in which case there is no obvious cytological difference between the sexes. Dipteran geneticists have termed the autosomal male-determining factors "*M* factors". Green[20] suggested that *M* factors at different loci in any one species were perhaps all actually the same gene, transposed to several different sites in the genome. The evidence now strongly favors this interpretation in several cases (discussed later under Diptera). This explains the exclusive nature of their occurrence in individual males, but makes the term "multiple sex factors" something of a misnomer.

This kind of sex determination may occur in other insects, especially where heteromorphic sex chromosomes have not been detected or do not occur regularly, but the necessary genetic evidence is lacking for other orders apart from Diptera.

E. HAPLODIPLOID SEX DETERMINATION

No general survey of sex determination in insects would be complete without mention of haplodiploidy, of which Hymenoptera are, of course, the leading exponents. Haplodiploidy may also be general to Thysanoptera, and is found in some species of Homoptera and Coleoptera (as well as occurring widely in mites and ticks). The genetic mechanisms involved have been the subject of much speculation (reviewed by Crozier[21,22]). Indeed, at first sight, it is difficult to see how any genetic mechanism at all can be operating, as haploid males simply

have half the female dose of all genes. However, several hypotheses based on multiple sex factors have been suggested, and one has some experimental foundation.

It has long been known that, in certain Hymenoptera, inbreeding results in diploid males,[23-25] indicating that not only haploids but diploid homozygotes are male. This can be explained if there are multiple sex factors ($S_1S_2S_3$...) — possibly alternative alleles at a single locus — segregating in opposition:

Females	Males
S_1S_2, S_1S_3, S_2S_3, ...	S_1, S_2, S_3, ... (if eggs unfertilized)
	or S_1S_1, S_2S_2, S_3S_3, ... (in inbred populations)

The sex factors appear to complement one another, and this has therefore been termed a complementary sex-determining mechanism.

While this explanation fits members of several groups of Hymenoptera very well (e.g., *Habrobracon, Apis, Neodiprion,* and *Solenopsis*), it cannot apply generally, because some other Hymenoptera inbreed considerably, yet fail to produce diploid males. To accommodate this problem, the hypothesis can be modified to involve multiple loci.[21] The theory is that diploids would then have to be homozygous at all loci in order to be male, and this would only be likely after long-term intensive inbreeding.

F. THE MOLECULAR BASIS OF SEX DETERMINATION

Thus there is a variety of ways in which sex can be determined in insects. Sex factors can apparently determine either maleness or femaleness, can be dominant or recessive in their action, can be single or multiple, and can occur on sex chromosomes or autosomes. How can all this be explained in molecular terms?

Our knowledge of the molecular biology of sex determination is almost entirely restricted to *D. melanogaster*, but at least in this one species some of the details of the mechanism are now worked out. The key gene is *Sexlethal* (*Sxl*), which is located on the X chromosome. This gene is essential for determination of females, but completely functionless in males, so that it can be eliminated by mutation without affecting the male phenotype. There is a maternal gene, *daughterless* (*da*), that has to be active for *Sxl* to function, because a mutation at the *da* locus causes mothers to produce only sons; however, *da* is not normally involved in sex determination. Activation of the female-determining function of *Sxl* in *Drosophila* is in fact dependent in some way on the ratio between the number of X chromosomes and the number of sets of autosomes (henceforth X:A). Thus, if X:A is 1.0 (as in diploid eggs with two X chromosomes), *Sxl* produces an active product that causes the embryo to develop as female, but if X:A is 0.5 (e.g., diploid eggs with XY or XO) then *Sxl* is silent and the embryo develops as male.

The genes repressing *Sxl* in male eggs have not been identified, but in *Drosophila* they must be located on the autosomes, because a genotype with one X chromosome and one set of autosomes (X + A) is female, whereas the addition of another autosome set (X + AA) results in a male. The molecular nature of the X:A signal is a source of continuing speculation.[26] Chandra[27] proposed that the normal, diploid forms of both sexes produce the same limited number of repressor (R) molecules; two X chromosomes can bind all the R molecules so that *Sxl* can be transcribed, but one X chromosome leaves sufficient R molecules to repress *Sxl*. One currently favored form of this hypothesis[28] has the X:A signal produced by two or more X-linked genes (e.g., *sis-a* and *sis-b* in Figure 6) whose products act as "numerator elements," and are titrated by certain autosomal products ("denominator elements"), so that a sufficient concentration of *sis* products to promote transcription of an active product by *Sxl* will only be achieved in females (Figure 6).[29,30]

Sxl regulates the differentiation of the female tissues, in *Drosophila* acting entirely through its control of a locus on chromosome 3, *transformer* (*tra*), which is also only functional in

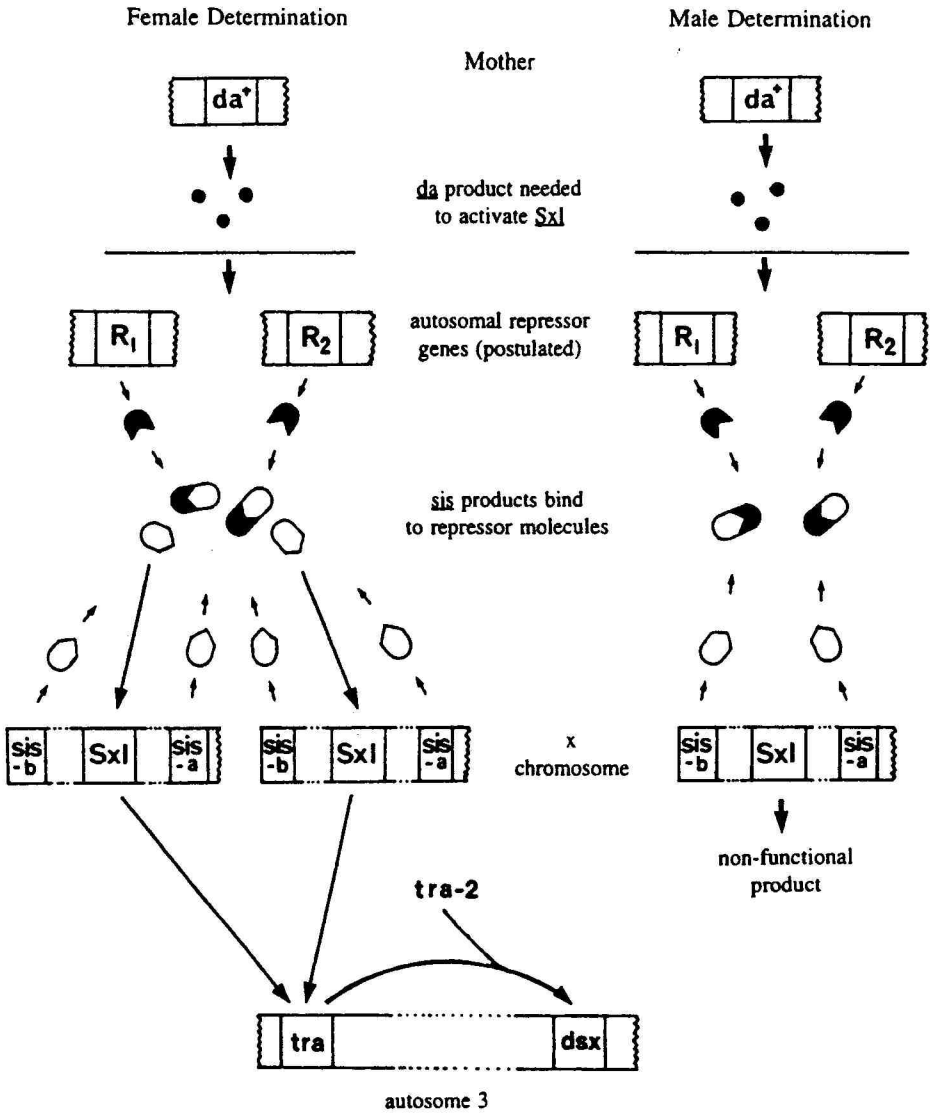

FIGURE 6. Simplified model of a possible mechanism for genetic control of sex determination in *Drosophila melanogaster*. Female determination and differentiation (left) depends on the product of the key gene *Sex lethal* (*Sxl*). The product of the maternal gene *daughterless* (*da*) needs to be present for *Sxl* to be active, but this is normally supplied to both male and female eggs. It is thought that unidentified autosomal genes produce a similar concentration of repressor molecules ("denominator elements": R) in both sexes. Female eggs, with two X chromosomes, produce twice as many "numerator elements" (i.e., products of X-linked loci such as *sis-a* and *sis-b*) as male eggs, so that there is an excess of unbound molecules to promote the female-determining activity of *Sxl*. The active product of *Sxl* influences the transcription of the product of the autosome 3 gene *transformer* (*tra*), which in turn acts on the *doublesex* locus (*dsx*: see text and Figure 7).

females. The *tra* product collaborates with the product of another gene (*tra-2*) to control the expression of another locus on chromosome 3, *doublesex* (*dsx*) (Figure 6). The *dsx* locus is active in both sexes and provides the double switch mechanism necessary to ensure that development proceeds only as either one sex or the other; it consists of two cistrons, dsx^m and dsx^f, only one of which functions in each sex. In female eggs (i.e., when both *tra* and *tra-2* are active), dsx^f is active and its products repress the male sex differentiation genes, whereas in male eggs the products of dsx^m repress female sex differentiation genes.

It is now known that regulation at all the three main stages occurs at the level of RNA splicing;[8] that is to say, the primary gene products are the same in both sexes, but they are "edited" by the splicing out of different sections (introns) of RNA to produce the male- and female-specific messenger RNAs (Figure 7). The male-specific messenger RNAs of both *Sxl* and *tra* include a stop codon which truncates the open reading frame so that the transcript is nonfunctional. This explains why mutational loss of these genes has no effect in males.

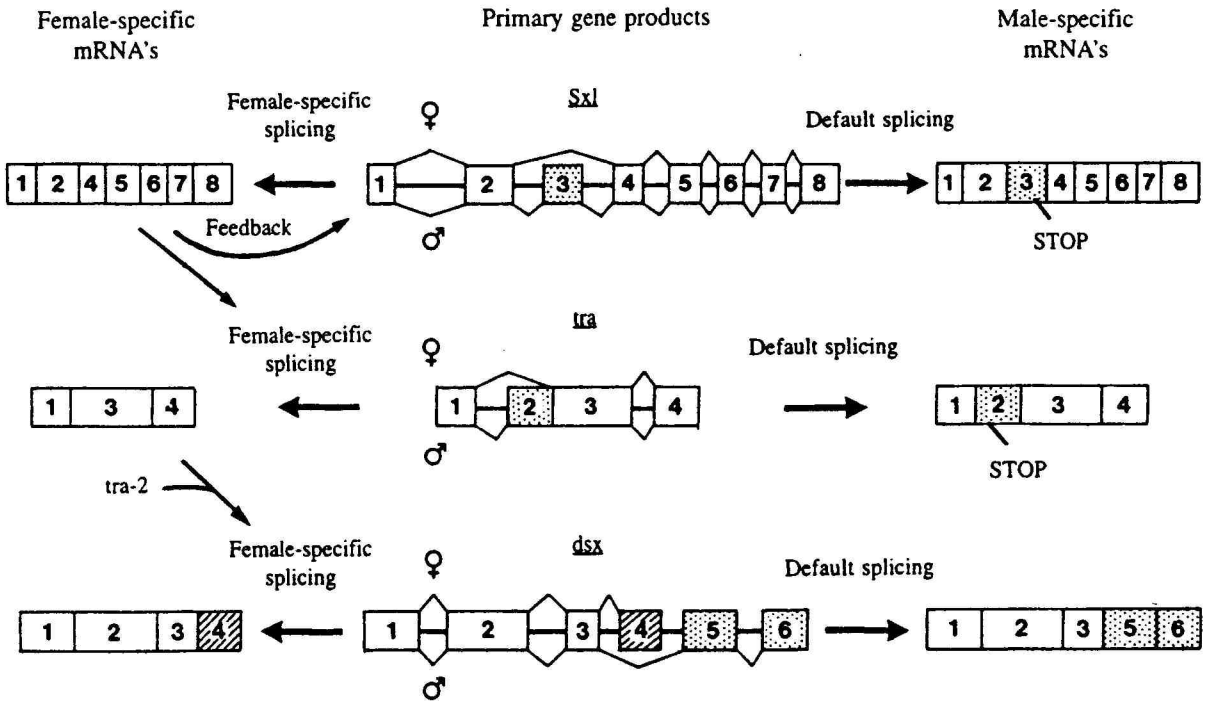

FIGURE 7. Production of sex-specific messenger RNAs (mRNAs) from the primary gene products of the *Sexlethal* (*Sxl*), *transformer* (*tra*), and *doublesex* (*dsx*) genes of *Drosophila melanogaster* by differential splicing. Primary transcripts of these genes are shown in the center; the boxes represent coding regions (exons), the horizontal lines joining them represent introns (which do not form part of an active product), and the female-specific and male-specific patterns of splicing are depicted, respectively, above and below the structures of the primary transcripts. The mRNAs generated by this process are depicted to left (female) and right (male) of the primary transcripts. In males the mRNAs result from the default pattern of splicing, which in the cases of *Sxl* and *tra* includes a stop codon rendering the mRNA nonfunctional. The female-specific product of *Sxl* regulates its own activity by positive feedback, and regulates *tra* activity by promoting the female-specific product of that gene, which in turn plays its part in directing the female-specific pattern of splicing of the *dsx* gene. (Adapted from Baker, B. *Annu. Rev. Genet.*, 17, 345, 1983.)

Thus, sex determination in *Drosophila* depends on a hierarchical system of regulatory genes. Can such a mechanism be generally applicable, given the apparently diverse systems of sex determination found in other organisms? Nöthiger and Steinmann-Zwicky[11] speculated on various mutations in the regulatory system that could, together with changes in the sex chromosomes, explain most of the variations observed in insects. They regarded the action of the double switch gene, *dsx*, as likely to be basic to the sex determination of all insects, and therefore not capable of functional mutation. They postulated that the primitive system in insects was probably represented by species in which the male is heterozygous at a single sex-determining locus. Using the notation introduced earlier in this chapter, in dominant-Y systems the sex factor s_2 acts as the male determiner by repression of the key gene *Sxl*, whereas the sex factor s_1 does not code for functional product, thus allowing *Sxl* to be active. If heteromorphic sex chromosomes are involved, then the male sex factor s_2 (or strictly S_2, since its effect is dominant) would be located on the Y chromosome, but it could be transposed to different locations in the genome, or copied to different locations to form a "multiple factor" (M) system. Recessive-X systems, which occur commonly and widely in insects, are explicable in terms of a genic balance as already discussed for *Drosophila*; the repressor gene is on an autosome, and *Sxl* is only activated if two X chromosomes are present, i.e., in individuals homozygous for s_1.

It is possible to interpret other, less common, types of sex determination in terms of mutations of the key gene *Sxl*, of its repressor, or of the maternal gene *da*. Some of these special cases will be referred to later in this chapter.

G. DOSAGE COMPENSATION

Organisms in which the Y has little or no homology with the X or does not exist at all (XO), have a gene dosage problem. An XX female has two copies of every X-linked gene, while an XY or XO male has only one copy. Mammals compensate for this by inactivation of one of the two X chromosomes in female somatic tissues. However, in *Drosophila*, where polytene chromosomes make it easy to study the level of transcriptional activity, dosage compensation is achieved in a different way. Both X chromosomes are active in female tissues, but the transcription rate is only half that of the single X chromosome in males, which produces just as much RNA as the two Xs in females put together.[7,31] The hyperactivity of X-linked genes in male *Drosophila* appears to be due to a set of genes (*msl*) which are inhibited when the key gene *Sxl* is active and are therefore only functional in males.[8]

The only other information on dosage compensation in insects is in Orthoptera. Rao and Ali[32] showed that both X chromosomes in hepatic cecal cells of female *Acheta domesticus* were euchromatic (i.e., transcriptionally active), and provided some evidence — using an indirect measure of transcriptional activity of unproven reliability — that the single X chromosome in the male may be hyperactive, as in *Drosophila*. On the other hand, females of the mole cricket *Gryllotalpa fossor* (=*africana*?) seem to have only one arm of one X chromosome transcriptionally active in hepatic cecal cells,[33,34] which resembles the system in mammals. However, there is evidence that activity or inactivity of the X chromosomes in Orthoptera may differ among tissues.[35]

In Lepidoptera, the limited evidence available from the differential activity of sex-linked loci suggests that members of this order may manage without a dosage compensation mechanism. Indeed, Johnson and Turner[36] suggested that in mimetic butterflies the dosage differential may be used to advantage, in order to limit expression of a polymorphism to the female sex.

III. SEX DETERMINATION IN DIFFERENT GROUPS OF INSECTS

A. APTERYGOTA

Of the four most primitive extant orders of insects, only the Collembola have been studied sufficiently to warrant generalization, and in these the male is the heterogametic sex and is normally XO,[37,38] but in Neanuridae, species with XO and others with XY are known.[39] Presumably the XY species are neo-XY, but there is no cytological evidence to confirm this. In *Neanura monticola*, with XO males, the X chromosome shows considerable polymorphism with large amounts of heterochromatin (probably repetitive, noncoding DNA) in high altitude populations.[39] In the Thysanura, *Thermobia domestica* possibly has X_1X_2O males.[40] In Protura, on the other hand, no instances of an XX/XO system have been reported; very few of the species examined had morphologically differentiated sex chromosomes.[41,42] No representatives of the Diplura seem to have been examined cytologically.

B. PRIMITIVE EXOPTERYGOTA

XX/XO sex determination predominates in the Odonata, possibly in the Ephemeroptera[43] (although these are poorly studied), and certainly in the main Orthopteroid orders (Dictyoptera-Phasmida-Orthoptera). Where an XX/XY system occurs in these groups, it is usually clear that it is a neo-XY system, formed by fusion of an X with an autosome (Figure 2), so that the neo-Y is homologous with a large part of the neo-X. In the anisopteran families of Odonata, for example (reviewed by Kiauta,[44,45]) most species are XX/XO, but there are apparent neo-XY systems in 15 species scattered through the families Gomphidae, Aeschnidae, Cordaliidae, and Libellulidae, representing about 4% of the dragonflies then studied. Species with neo-XY generally have, as might be expected, one less autosome pair than related species with an XO

system; e.g., *Aeshna crenata* has 2n = 28 and XO males, whereas *A. grandis* has 2n = 26 and neo-XY males.[46] Kiauta,[44] followed by Tyagi,[47] explained an evolutionary decrease in the number of autosomes in the family Gomphidae as a succession of fusions and translocations between the neo-Y, the neo-X, and autosomes, the outcome of each step being a secondarily derived XO system with one fewer autosome pairs. However, there is no clear cytogenetic evidence that the sex chromosomes are involved in these changes of karyotype.

C. THE ORTHOPTEROID ORDERS

In the Plecoptera, which are generally thought to be an orthopteroid order that retains primitive features, several species in different genera have XO males, always with a very large metacentric X chromosome that moves in a highly characteristic way in the first meiotic division.[48] XY males (presumably neo-XY) are only recorded for one species (*Perla* (=*Paragnetina*) *immarginata*), but some species of *Perla* have a multiple X chromosome system apparently derived from XX/XO, males being X_1X_2O, and in *Perlodes* there are three species known with $X_1X_2X_3O$ males. The two or three Xs in these species are much smaller than the single X of XO and XY males in related species, and associate together in the first meiotic division. Their mode of origin is a mystery, as there is no simple way in which a large metacentric chromosome can give rise to several smaller elements.

The cytogenetics of the other orthopteroid orders has been comprehensively reviewed by Hewitt[49] (Orthoptera) and White[50] (Grylloblattodea, Dictyoptera, Isoptera, Phasmida, Dermaptera, and Embioptera), so information about the sex chromosome systems of these groups will only be summarized here and up-dated. Dictyoptera (Blattodea + Mantodea), Phasmida, Orthoptera, and Embyoptera all seem to be primitively XX/XO, whereas in Isoptera and Dermaptera XX/XY predominates and is possibly the primitive condition.

In Blattodea (cockroaches), males seem to be invariably XO where both sexes have been karyotyped.[51] White[50] suggested that this stability of the sex determination system could be due to the fact that the X is almost always metacentric, and therefore not so readily available for centric fusion with an autosome to generate a neo-XY system. Yet it is difficult to see why this argument does not equally apply to Phasmida, which also have a metacentric X yet frequently develop a neo-XY system.

Isoptera (termites) are generally thought to have arisen from primitive Blattodea but, whereas the most primitive cockroach examined cytologically has XX/XO,[52] the most primitive extant termites seem to be mainly XX/XY.[53] Nevertheless, some species do have XX/XO,[54] and fusions and translocations between sex chromosomes and autosomes are so common in termites[53,55] that XX/XO could still be the primitive condition.[56]

Several species of Kalotermitidae in southern U.S. and the Caribbean form remarkable chains or rings of up to 19 chromosomes in male meiotic nuclei,[57-60] often involving more than half the total chromosome complement. The chains are thought to be due to a series of reciprocal translocations involving both the sex chromosomes and the autosomes of one chromosome set, these changes being restricted entirely to the male line, so that all the chromosomes involved function together as a multiple Y chromosome complex (Figure 8). Females are structurally homozygous and form normal bivalents at meiosis. The genetic consequences of such an arrangement are quite profound; for example, they restrict many alleles to males and increase the genetic similarity of offspring to the same-sex parent and to same-sex siblings. The idea that this unusual system has played a significant part in the development of eusociality in termites[61] is, however, somewhat undermined by the fact that the most extreme rearrangements are found in only a few of the more primitive termites.

Mantodea also seem to have an XX/XO mechanism with a metacentric X chromosome as the primitive condition, but members of the largest subfamily Mantinae consistently have an $X_1X_1X_2X_2$:X_1X_2Y mechanism that has aroused considerable interest among cytogeneticists. White[62] proposed that this was derived from XX/XO by translocation between the X and a metacentric autosome (Figure 4), and all subsequent evidence has been consistent with this

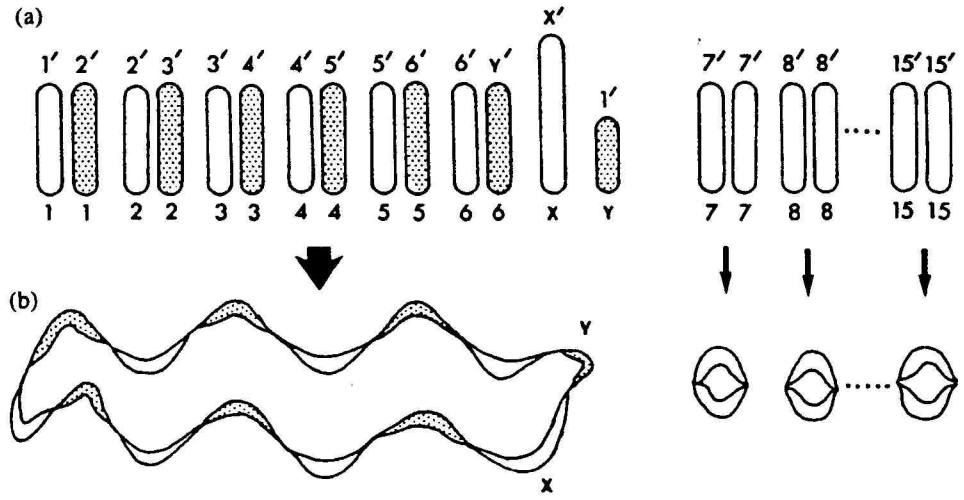

FIGURE 8. Diagram illustrating how a series of reciprocal translocations, involving one member of each of six autosome pairs and the Y chromosome (a), could lead to a ring of linked chromosomes in male meiosis of the termite *Incisitermes schwartzi*. The reciprocal interchange set is stippled, and only occurs in males. Chromosome pairs not involved in translocations (right) form normal bivalents. Sizes of chromosomes are arbitrary; the X and Y chromosomes have not actually been distinguished from the autosomes or from each other in this species. (Adapted from Syren, R. and Luykx, P., *Nature (London)*, 266, 167, 1987.)

hypothesis.[50] All Mantinae have a remarkably consistent chromosome complement, with 2n (male) = 27 (only one exception is known — see below). Presumably the X_1X_2Y system had a single origin in the evolution of this subfamily, and some of the species with X_1X_2Y males currently placed in other subfamilies are perhaps wrongly classified. However, the African mantid genus *Compsothespis* has an X_1X_2Y system with much smaller sex chromosomes, and 2n (male) = 23; at least in this case, an independent origin seems likely (see White[50] for further details). It is not at all apparent why this system has proved so successful for mantids. The mechanism itself does not seem very efficient; complications often seem to arise in correctly forming an X_1X_2Y trivalent in the first division of spermatogenesis, and consequently in correctly segregating the X_1 and X_2 into one daughter spermatocyte and the Y into the other. Callan and Jacobs[63] showed for *Mantis religiosa* that the first meiotic division of those spermatocytes that fail to form the X_1X_2Y trivalent is inhibited, thus preventing the formation of aneuploid sperm. Liebenberg et al.[64] reported a single male of *Polyspilota aeruginosa* (Mantinae) with 2n = 28 instead of the usual 2n = 27, and an $X_1X_2Y_1Y_2$ mechanism. The origin of the extra Y (neo-Y) in this one aberrant case is unclear.

White[50] listed 57 species of Phasmida (stick insects) with identified sex chromosomes, of which 49 are reported to have XO males — undoubtedly the primitive condition — and 7 species (in 6 separate genera) have a neo-XY system arising through fusion of an X with an autosome (see Figure 2). The one other species studied, *Didymuria violescens*, occurs in Southeast Australia, where it has at least 10 chromosomal races, occupying contiguous distribution areas, and including both XO and neo-XY forms.[65] Several independent origins of a neo-XY system can be traced from XO ancestors.[50]

In the well-known parthenogenetic stick insect *Carausius morosus*, males and masculinized females (intersexes or sex mosaics) appear occasionally in laboratory cultures, and their numbers can be enhanced by various treatments, e.g., subjecting the eggs to high (30°C) temperature,[66] centrifuging the eggs,[67] X-irradiating egg or oocytes,[68] or injecting the mother with pterine derivatives.[69] Females and masculinized females have three metacentric chromosomes that are regarded as sex chromosomes because of their behavior in meiosis. Males lack one of these sex chromosomes or a segment of one of them.[70] The method of sex determination is difficult to work out because the female karyotype is highly aberrant due to its long history of parthenogenesis. It has been suggested that *C. morosus* originated as a triploid or tetraploid.[71] However, whatever their origins, both sex chromosome and autosome complements

are now aneuploid, and cannot be regarded as comprising any particular number of chromosome sets. Male determination presumably occurs because of a change in the genic balance between factors on the sex chromosomes and on the autosomes (so that, assuming that the molecular model established for *Drosophila* applies, the key female-determining gene *Sxl* is repressed). It is not clear why intersexes, which retain the female karyotype, arise under certain conditions; one possibility is that high temperatures, etc. prevent splicing of female-specific messenger RNAs. General inactivation of the sex chromosomes by heterochromatinization has been suggested[70] to function in sex determination, but such heterochromatinization has only been observed in germ-line (spermatogonial) interphase nuclei, and it is not known whether it occurs in embryonic somatic cells.

Only eight species of Embioptera have been studied cytologically (four in each of the families Oligotomidae and Embiidae), and all have an odd number of chromosomes in male somatic cells, indicating that sex determination is probably XX/XO, with the X chromosomes large and metacentric.[50] Nothing is known about sex determination in Zoraptera.

Hewitt[49] comprehensively reviewed the extensive cytogenetic studies that have been carried out on the Orthoptera proper (Saltatoria). Since Hewitt's review, there have been significant contributions on the sex chromosome systems of neotropical Acridoidea (about 200 species[72]), the acridoid subfamilies Catantopinae,[73] and Pamphaginea,[74] Indian Orthoptera (30 species[75]), and certain Tettigonoidea.[76-78] XX/XO sex determination is found in the great majority of species in all subdivisions of the order, both primitive and advanced, and is undoubtedly the primitive condition for the Orthoptera as a whole. The only exception is the relic group Grylloblattodea, with XY males in the only two species studied,[50] but in the face of all the other evidence, this must be regarded as a derived state. About 8% of species have XX/XY or $X_1X_1X_2X_2/X_1X_2Y$ systems, which occur in every major subdivision of the group and are usually clearly evolved secondarily from an XX/XO condition by centric fusion (Figures 1, 2). Two cases are known, one in Eumastacoidea ("Morabinae species P45b")[79] and the other in Tettigonoidea (*Callicrania seoanei*)[76] of the neo-X being formed by "tandem fusion" of an autosome to the centromeric end of the original X. In both these cases, the neo-Y forms a terminal connection with the neo-X at meiosis, and this neo-XY bivalent divides equationally at first meiotic division, so that the X and Y do not segregate until the second division ("postreductional meiosis"). The mantid type of origin of an X_1X_2Y system, directly from XX/XO by translocation between an X and an autosome, is not known to occur in Saltatoria, perhaps because autosomes in this order are predominantly acrocentric,[9] making centric fusions a more likely occurrence.

The neo-X produced by centric fusion between an acrocentric X and an acrocentric autosome is likely to be large and metacentric, and the neo-Y (the original autosome) is acrocentric (see Figure 1); the majority of cases of neo-XY systems in Saltatoria have sex chromosomes of this form (see Table 8 in Hewitt[49]). Likewise, neo-X_1X_2Y males produced as a result of a Y-autosome fusion have a metacentric X_1 and Y and an acrocentric X_2 (Figure 2); again, the majority of X_1X_2Y systems in Saltatoria conform to this pattern. This may reflect the recent origin of many of these systems because, once a neo-Y is formed, it is subject to very different evolutionary pressures from the original autosome. Several species have been studied that have both XO and neo-XY populations;[73,77,79-82] presumably the neo-XY system is only very recently established in such populations, and in some cases the early stages of differentiation of the neo-Y from its homologue, now part of the neo-X, can be observed. The neo-Y may acquire heterochromatic segments, and pairing between the neo-X and neo-Y may become restricted to terminal regions, so that crossing-over is limited, paving the way for further differentiation of the genetic role of the neo-Y from that of its former homologue.

In time, as discussed earlier, the neo-Y is likely to degenerate; an example of this may be the Gryllacridoid genus *Dolichopoda*, where the "neo"-XY system is possibly as old as the genus itself, and all species studied have a large metacentric X and a small dot-like Y.[83] However, no instance has yet been identified in Orthoptera of the complete loss of a neo-Y,

to revert to an XO system, which suggests that the neo-Y may acquire and retain some functional male-linked loci.[49]

The earwigs (Dermaptera) seem to stand somewhat apart from the other orthopteroid orders, and this is reflected in their chromosomes, which have diffuse centromeric activity like those of Hemiptera, and in their sex determination system, as the primitive condition for the group seems to be XX/XY rather than XX/XO. Only two species with XO males are recorded, belonging to different families.[84] Multiple sex chromosomes are very common, occurring in about half the species that have been karyotyped, with similar frequency of incidence in all families. Multiple Xs have probably arisen by simple dissociation of the existing X chromosomes, as in other insects with holocentric chromosomes (Figure 5). They form a close cluster on the spindle at first meiotic division, and all move together to one pole, while the X moves to the opposite pole. The ubiquitous earwig *Forficula auricularia* is unusual in having two alternative Y chromosomes, one of which ("Y_2") is mitotically unstable so that it tends to accumulate in number, and individual males may have up to four copies ($XY_2Y_2Y_2Y_2$). Mosaic males have been recorded with different numbers of Y chromosomes in the cells of each testis.[84]

D. THE HEMIPTEROID ORDERS

The Psocoptera are generally regarded as close to the basal hemipteroid stock, and all the 32 species so far examined cytologically[85] seem to have XX/XO sex determination. Nothing is known about the sex-determining mechanisms of biting and sucking lice (Mallophaga and Siphunculata), as no sex chromosomes have been identified in any of them. When a female human louse (*Pediculus humanus*) is mated with a single male, the sex ratio of the progeny is strongly biased toward one or other sex, and unisexual broods are common.[86] Contrary to White,[9] no information is available about the progeny of females mated more than once, and it seems likely on the available evidence that maternal factors are involved in the determination of sex in lice, as in certain Diptera (e.g., *Chrysomya*).

Most species of Heteroptera have XX/XY sex determination,[87] but there are some groups — e.g., 124 species in the related families Coreidae and Alydidae — that are almost exclusively XX/XO.[88] XO males also predominate in the supposedly more primitive Heteroptera (Gerromorpha; but see Calabrese and Tallerico[89]), and Ueshima[87] concluded that the XY system in Heteroptera, despite its widespread occurrence, is derived from a primitive XO condition. Nokkala and Nokkala,[90] on the other hand, argued that XY was ancestral. Clearly, XY systems are ancient and well-established in terrestrial Heteroptera; the X and Y chromosomes generally show little or no evidence of the homology expected of a neo-XY system and undergo a characteristic pattern of meiotic behavior in which they usually segregate at the second division (for details, see White[9] (pp. 620–621) or Ueshima[87]). The scattered occurrence of XO species within genera must surely be due to secondary loss of the Y chromosome, and such loss may have occurred early in the evolution of many families of terrestrial Heteroptera. However, this does not rule out the possibility that the common ancestor of all Heteroptera was XO, as in Psocoptera, and that XX/XO sex determination may be the primitive condition in some families of Gerromorpha. The problem can only be resolved when the cytology of members of the most primitive groups, Enicocephalomorpha and Dipsocoromorpha, now thought to have a sister-group relationship with all other Heteroptera,[91] as well as of the relic family Peloridiidae (suborder Coleorrhyncha), have been examined. The only information for these groups so far is for one species of Dipsocoromorpha, males of which were tentatively recorded as XO.[92] Multiple sex chromosomes are common in Heteroptera, and may be derived from either XY or XO systems. Apparently they are in most cases due to dissociation of the X chromosome into two or more smaller parts, which group together on the spindle of the second meiotic division and move *en bloc* to one pole (see Figure 5). In some species, the number of X chromosomes varies; the best-known example is the bedbug *Cimex lectularius*, where the number of separate X elements varies from 2 to 15.

Messthaler and Traut[93] showed that the Y chromosome was heterochromatic and therefore transcriptionally inactive in all stages of spermatogenesis of the milkweed bug, *Oncopeltus fasciatus*. Despite the reservations of Thomas,[94] there can be little doubt that the Y chromosome in Heteroptera is genetically inert, and that sex determination is based, as in most insects, on a "recessive-X" (i.e., genic balance) system. Otherwise it would be impossible to explain how the secondary loss of the Y chromosome could occur in so many groups without concomitant loss of genetic viability.

Sex determination in Homoptera-Auchenorrhyncha is predominantly XX/XO.[95,96] A few species with XY males occur within genera and subfamilies that are otherwise exclusively XO, and in such cases it is often clear that the Y is a neo-Y; i.e., the homologue of an autosome that has recently fused with the X. Such a neo-Y pairs with the autosomal part of the neo-X in meiosis and segregates from it at the first division.[97] In several species of *Oncopsis*, both XO and neo-XY males occur in the same or different populations; the XY state results from fusion of the X chromosome with a different autosome in each species.[98]

The Homoptera-Sternorrhyncha include some of the most specialized hemipteroid families, and the basic system of sex determination is often obscured, especially in groups with well-developed parthenogenesis. The Psylloidea are the least reproductively specialized, and here again sex determination is predominantly XX/XO. Only three species with XY males have been found in a total of 39 species examined, all apparently recently derived from XO by X chromosome-autosome fusion.[99-101] The Aleyrodoidea have received very little attention from cytogeneticists. On the basis of early cytological work on three species,[102,103] and the observation that males are only produced in laboratory populations by unmated females, the general presumption is that all male aleyrodids are haploid.[9] It would be preferable to have this confirmed for more species before assuming that haplodiploidy is of general occurrence in this group. The factors invoking male determination are unclear, but the cytological mechanism in those species studied seems to be the same as in Hymenoptera, with meiosis replaced by a single mitotic division, each primary spermatocyte giving rise to only two spermatids. Populations of *Trialeurodes vaporariorum* seem to have an approximately 1:1 sex ratio in field populations in both Europe and North America,[104] which is unusual for a haplodiploid system. However, thelytoky is a complicating factor in interpreting sex ratios in this species. The populations originally introduced from North America to England consisted almost exclusively of thelytokous females,[103] and it is not known what proportion (if any) of females reproduce thelytokously in present-day populations. In *Bemisia tabaci*, which has not been studied cytologically, thelytoky is unknown and the number of males produced seems to be temperature dependent.[105]

It is impossible to do justice here to the remarkable sex determination systems of scale insects (Coccoidea), and for details the reader is referred to the authoritative reviews by Nur.[106,107] All the different systems are believed to have evolved from an ancestral XX/XO system which is still found in some members of the more primitive families (Ortheziidae, Margarodidae, Phenaeolaechiidae). Some of the margarodids (*Icerya* and four closely related genera) have evolved male haploidy, and in some species of *Icerya* there is the further development of hermaphroditism, with morphologically female individuals maturing haploid sperm and diploid ova in an ovotestis. In hermaphrodite *Icerya*, fertilization is usually between eggs and sperm of the same individual; nevertheless, some eggs apparently remain unfertilized and give rise to functional haploid males. In all the more advanced families of Coccoidea, the paternal set of chromosomes is rendered inactive in most tissues by heterochromatinization during the development of male embryos (the "lecanoid" and "Comstockiella" systems; see Nur[106]). It seems that sex in these families is determined maternally rather than by the genotype of the zygote, because the sex ratio is greatly affected by the age of the female at mating and by environmental conditions such as temperature.[107] It is not clear, however, whether the inactivation occurs after, and as a consequence of, the embryo already having

been determined as male, as in Sciaridae (see Diptera, below), or whether the inactivation process itself provides the mechanism for male determination.[107]

Bull[10] pointed out that the evolution of these advanced coccoid systems from XX/XO is something of a mystery, because the heterochromatinized paternal chromosomes are eliminated in spermatogenesis, so that all sperm carry only the maternal genome. There is thus no genetic polymorphism among sperm to serve as a basis for sex determination, which effectively means that the advanced coccoid systems can never have coexisted with a system such as XX/XO, and must therefore have evolved through a form of sex determination without male heterogamety. There are a few coccid species (e.g., *Lachnodius eucalypti*) without identifiable sex chromosomes, and which do not undergo heterochromatinization of one chromosome set in the male (*2N-2N* of Nur[106]). Nur thought that these were probably derivatives from forms with heterochromatinization, but Bull's argument makes it more likely that they are representative of this intermediate stage, evolved from XX/XO prior to the origin of heterochromatinization, which is in line with the original views of Brown.[108] Haig has developed a model for the evolution of the advanced coccoid systems based on sex ratio theory.[109]

Aphids (Aphididae) all have XX/XO sex determination. An XX/XY system would be an impossibility for these cyclically parthenogenetic insects, because most species exist through the summer as all-female, thelytokous populations, and during this period the Y chromosome would have "nowhere to go". Aphids produce males parthenogenetically. To develop as XO males, oocytes have to lose half the sex chromatin of the parent female. This is achieved in a single egg maturation division, as in the thelytokous production of females, but the X chromosomes pair during prophase[110] and then undergo a sort of "mini-meiosis" on their own, first separating the products of pairing and then dividing equationally with the autosomes, all on the spindle of the single maturation division.[111,112] This peculiar cytological mechanism for male determination is of special interest because it is normally triggered by environmental conditions and mediated by a low level of juvenile hormone in the haemolymph; males can be induced by treatment with precocene, which destroys the corpus allatum, and inhibited by the juvenile hormone analogue kinoprene.[113] The environmental factors are normally photoperiod (actually the length of the dark phase) and temperature in Aphidinae, but may be nutritional in other groups, and in some species, males appear spontaneously or after a genetically programmed number of thelytokous generations.[114]

The spermatogenesis of aphids is also relevant to their sex determination, because the fertilized eggs must all develop as thelytokous females, so all the sperm from XO males must carry an X chromosome. This is achieved by a peculiar first meiotic division in which the X is stretched on the spindle before passing into one of the daughter spermatocyte nuclei, after which the daughter nucleus without an X degenerates.[115]

Multiple X chromosome systems occur in some aphids, apparently as a result of dissociation of the original X, and the separate elements all behave in the same way in meiosis.[116,117] The greenideine species *Schoutedenia ralumensis* (=*lutea*) has what was presumably originally an $X_1X_1X_2X_2/X_1X_2O$ system, but it has become modified in a remarkable way by consistent association or temporary fusion of one member of an autosome pair with X_1 and the other member of the same pair with X_2.[118] Male determination necessarily retains both these autosomal homologues (AA), so males receive the two elements X_1 + A and X_2 + A. One of the X chromosomes (it is not clear whether it is X_1 or X_2) then has to lose its connection with the autosome at anaphase I of spermatogenesis, so that males can transmit one X + A and one X to the next generation (Figure 9). How this peculiar system evolved as a stable mechanism for sex determination is something of a mystery.

Multiple X chromosome systems with X_1X_2 males also occur in the primitive aphidoid families Phylloxeridae and Adelgidae (see Blackman[119] for review), and show some unusual features in the few species studied. In particular, there seem to be species in each group which have evolved a potential for male-linked inheritance "by proxy," which overcomes the total

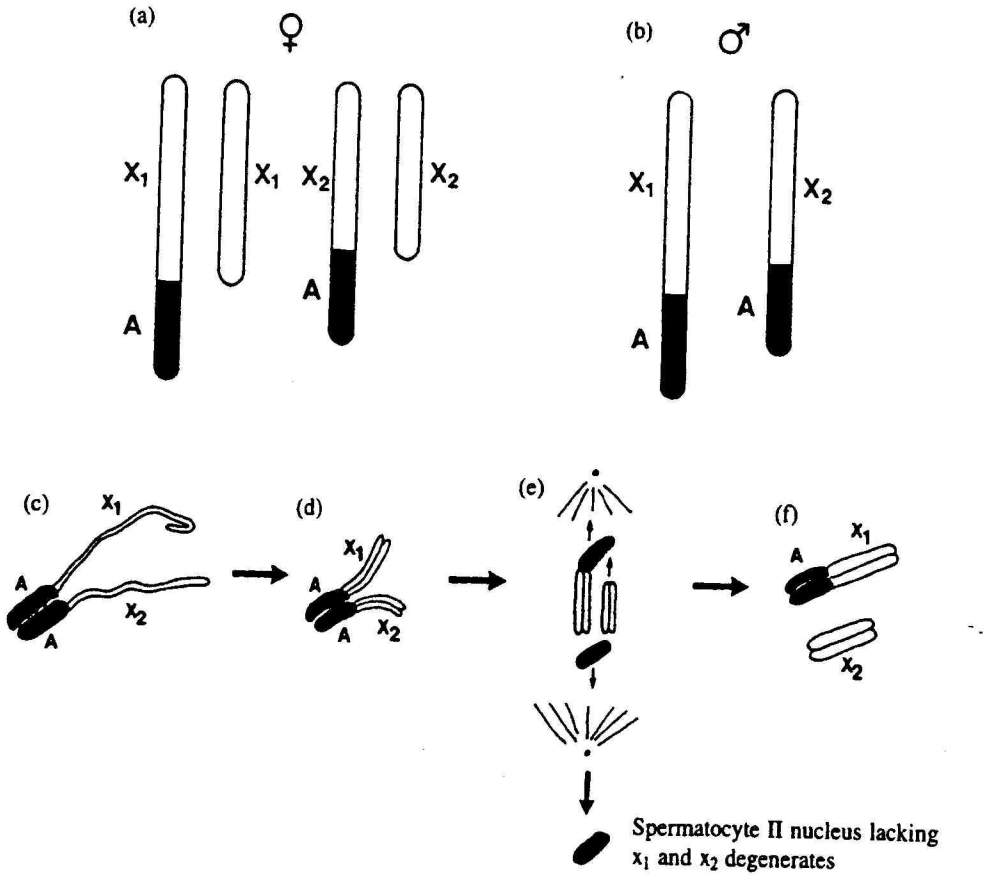

FIGURE 9. Sex chromosome-autosome associations in the aphid *Schoutedenia ralumensis* (=*S. lutea*). For simplicity, only the X chromosomes and the pair of autosomes (AA) associated with them are shown. Female somatic cells (a) have four long chromosomes of unequal length, representing X_1, (X_1 + A), X_2 and (X_2 + A). Male somatic cells and spermatogonia (b) have the longest two chromosomes, which are (X_1 + A) and (X_2 + A). In spermatogenesis, at prophase of the first meiotic division, the autosomes attached to X_1 and X_2, being homologous, pair in parallel (c,d). When the cell divides (anaphase), either X_1 or X_2 loses its connection with the autosome (e; shown here as X_2, but it is uncertain which). The lost autosome passes into one daughter cell which lacks both X chromosomes and degenerates. The other daughter cell has both X chromosomes, with one autosome still attached to one of them (f); it divides equationally to give spermatids with the same chromosome constitution. Presumably, for the system to be stabilized, oogenesis must somehow result in oocytes with the complementary arrangement; i.e., if sperm have (X_1+ A) and X_2, oocytes will have X_1 and (X_2 + A). It is not known how this is achieved. (Based on Hales, D. *Chromosoma* 98, 295, 1989.)

absence of males during the parthenogenetic (thelytokous) part of the life cycle, by having two cytologically distinct types of all-female line; one leading eventually to male production and the other to sexual females. In *Phylloxera caryaecaulis*, studied by the pioneer cytogeneticist T. H. Morgan,[120] one member of the smaller "pair" of X chromosomes seems to be limited to the male-producing line, and behaves differently from the other in its pairing relationships during sex determination and spermatogenesis (Figure 10). In the adelgid *Gilletteella (=Adelges) cooleyi*, Steffan[121] found one member of the longer pair of X chromosomes dissociated into two parts in about 50% of thelytokous females, and in the somatic cells of males, but not in sexual females. Further work is needed on these groups to confirm and extend these findings.

The last hemipteroid order to be considered is the Thysanoptera (thrips), both suborders of which (Terebrantia, Tubulifera), on the basis of the few species that have been studied cytologically, have haploid males.[122,123] The cytological mechanism involved is not very clear, but seems to differ from that of Aleyrodoidea and Hymenoptera, and must be independently derived. Instead of meiosis being replaced by a single mitotic division, as in other insects with haploid males, two meiotic divisions are retained; the first is apparently equational, giving rise to two similar-sized spermatocytes, but the second produces one large functional spermatid

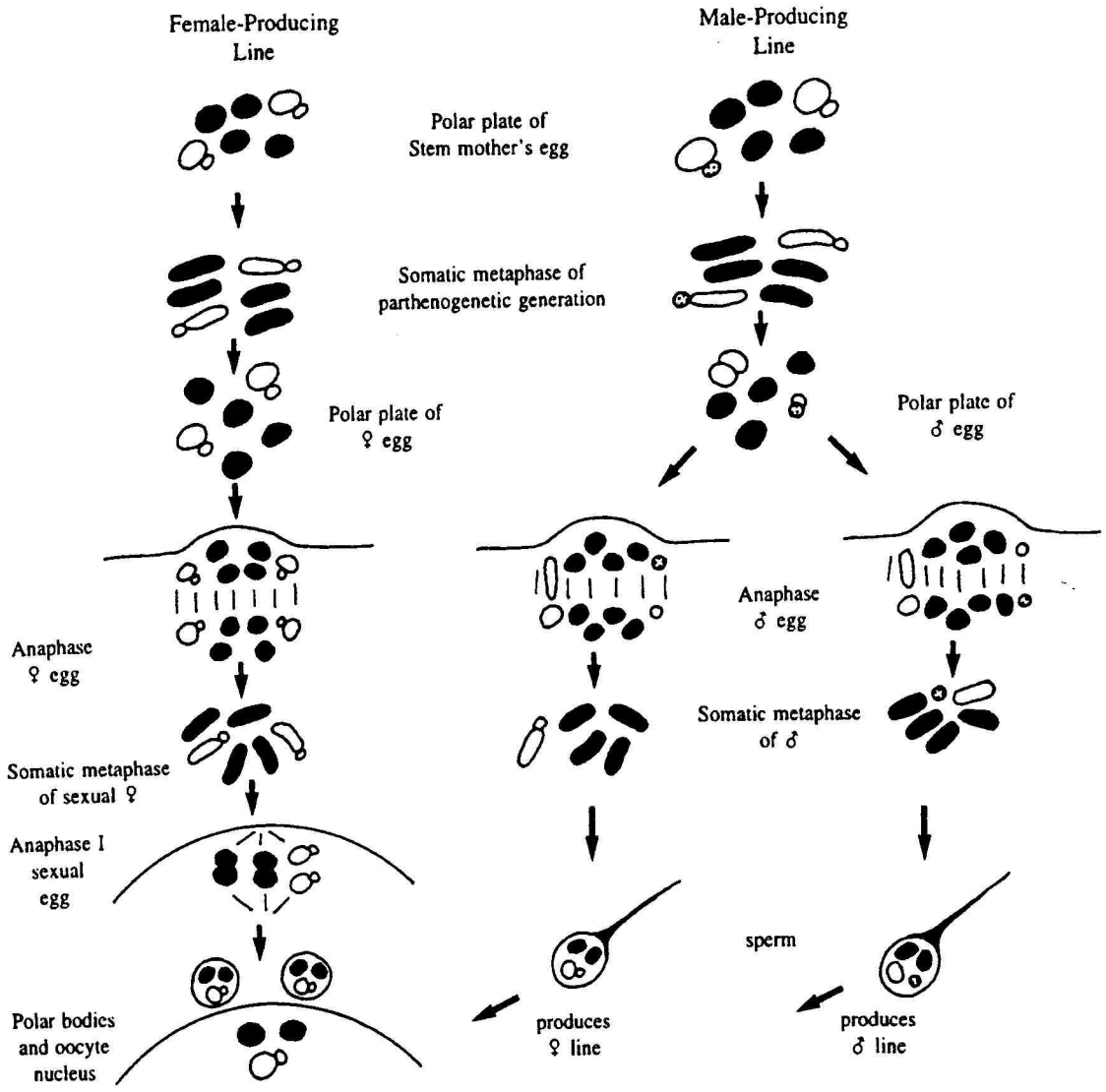

FIGURE 10. Chromosome cycle of *Phylloxera caryaecaulis*, redrawn from Morgan.[120] Autosomes are shown black, X chromosomes white, except for one member of the smaller pair (X_2) in the male line, which is stippled to show its differential behavior and possible role in sex determination. In the line leading to production of sexual females (left), small and large X chromosomes seem to be consistently associated, in the somatic cells of both parthenogenetic and sexual females and throughout oogenesis. In the line leading to male production (right), the small and large X chromosomes are likewise associated throughout the parthenogenetic phase, but during maturation of eggs destined to become male, the Xs exchange partners, so that the two large X chromosomes form one pair, and the small Xs another. Consequently, at maturation division of male eggs, the small and large X chromosomes segregate from each other independently. Males have X_1X_2O; half of them apparently have X_1 and X_2 associated together as in females, and half have them separate. Sperm with separate X_1 and X_2 are believed to give rise to the parthenogenetic line that will produce the males of the next bisexual generation.

and one much smaller one that rapidly degenerates.[122] As in the Aleyrodoidea, the factors invoking male determination are unclear; sex ratios show considerable variation within and between species,[124] but the interpretation of these in genetic terms is complicated by the occurrence of thelytokous parthenogenesis in many of the best-studied species.[125] In *Elaphrothrips tuberculatus*, females have unisexual broods, the males being produced viviparously and the females oviparously; more males seem to be produced when the offspring are larger and fitter in the favorable nutritional conditions of spring.[126]

E. NEUROPTEROIDEA AND COLEOPTERA

The Neuropteroidea (Megaloptera, Raphidioptera, and Plannipennia) are generally regarded as an early branch in the phylogeny of the endopterygote insects, but no species have

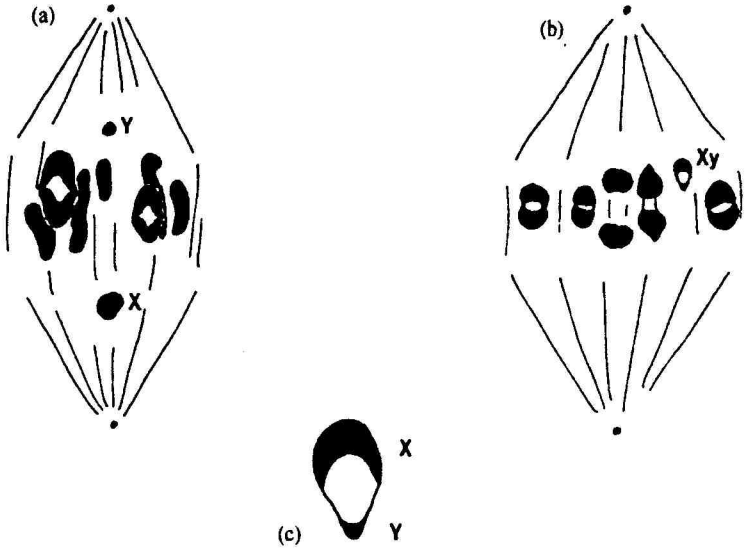

FIGURE 11. Diagrammatic drawings of first meiotic metaphase of male of (a) the neuropteran *Macroneurus appendiculatus*, showing "distance pairing" of X and Y chromosomes, and (b) the megalopteran *Neohermes filicornis*, showing X and Y forming a bivalent like the "parachute bivalent" (Xy_p) of Coleoptera. Structure of the parachute bivalent is shown in (c). (Based on Hughes-Schrader, S. *Chromosoma*, 81, 307, 1980.)

been found with XX/XO. Almost all species studied seem to have XX/XY sex determination, with a few showing multiple X systems. The X and Y chromosomes of Raphidioptera and Plannipennia (=Neuroptera *sensu stricto*) behave in a very consistent fashion during spermatogenesis (Figure 11a). They are both small chromosomes that apparently lack any homology, because they never pair to form a bivalent in the first meiotic division, and regularly take up positions in opposite halves of the spindle before segregating into the daughter spermatocytes.[9,127] In the two species of Megaloptera that have been studied, however, the X and Y chromosomes form a bivalent that positions itself with the autosomes on the equator of the spindle and segregates synchronously with them at the first meiotic division.[128] The Y chromosome is much smaller than the X, and in one species the bivalent looks very like the "parachute" bivalent (Xy_p) found in Coleoptera (Figure 11b,c, and see below).

Thus, the sex chromosome systems of the Neuropteroidea seem to provide useful phylogenetic evidence pointing to a sister-group relationship between Raphidioidea and Plannipennia, and also supporting the often-held view (e.g., Henning[129]) that the Megaloptera are the sister group to the Coleoptera.

The Coleoptera show a great diversity of sex chromosome systems, although the underlying genetics of sex determination may well be far less variable, and is likely to be based on a recessive-X mechanism, except where male haploidy has evolved. Coleopteran cytogeneticists have accumulated information about the sex chromosome systems of over 2500 species. Fortunately, the comprehensive reviews by Smith and Virkki[130] and Virkki[131] mean that only a brief overview and some updating are necessary here.

The peculiar symbols used in the literature on beetle sex chromosomes are somewhat daunting to the nonspecialist, but can be simply explained. They symbolize the appearance and behavior of the sex chromosomes in the first meiotic division of the male beetle. Sex chromosome symbols are written together if there is any sort of pairing between them to form a bivalent (e.g., XY), but separated by a plus sign (e.g, X+Y) in the much rarer cases where they do not pair. The Y chromosome is usually very small in Coleoptera, and this is indicated by writing Xy instead of XY. In most Polyphaga with Xy, the minute Y is attached by both its arms to the larger X, so that it resembles a parachutist suspended below the "canopy" formed by the X (Figure 11c). The formation and structure of the parachute has recently been studied by silver staining;[132] it is believed to have a role in assisting the regular segregation of the X and Y at first meiotic division. When the Xy bivalent takes this form, then a subscript

"p" (for parachute) is added: Xy_p. XX/XO systems in Coleoptera are represented by a single X, rather than as XO. Systems with multiple small Y (=y) chromosomes involved in a single parachute are written Xyy_p, $Xyyy_p$, etc.

Two main types of neo-XY system occur in beetles; those with a large Y, probably derived from an XO system by X-autosome fusion (e.g., Figure 2), and those where an autosome has apparently undergone a reciprocal translocation with either the X_p or the y_p of an Xy_p system to give an "$X_p$neoX-neoY_p", or some other complex system in which the original parachute has elements (neo-X, neo-Y) associated with it in the first division of meiosis.[131,133]

The more primitive beetles (Adphaga) differ from the Polyphaga in that Xy_p systems are virtually absent except in a few Dytiscidae (records of Carabidae with Xy_p are apparently questionable[134]). XX/XO is most frequent in Adephaga, occurring in about 53% of species, with 29% having XX/XY (or XX/Xy).[134] XY systems predominate, however, in the carabid genus *Bembidion* (176 out of the 205 species examined[135]). Tiger beetles (Cicindelidae) mostly seem to have multiple X systems, with 2, 3, or 4 X chromosomes.[136]

More than half of over 2000 species of Polyphaga examined cytologically have Xy_p sex chromosome systems, which are well represented in every major family, and are generally thought to be the ancestral condition for the whole suborder. Whether Xy_p is the primitive condition for all beetles is not quite so clear, because of its rarity in Adephaga, although the recent finding of a sex parachute in a megalopteran makes it more likely. Only single species have been examined in each of the two primitive beetle suborders Archostemmata and Myxophaga, and they may both be untypical.[130,137]

Since Virkki's 1984 review,[131] there have been significant studies on the sex chromosome systems of Chrysomelidae,[138,139] Histeridae,[140] Indian Staphylinidae,[141] Indian Curculionidae,[142] 32 other Indian beetle species,[143] Tenebrionidae,[144] Bruchidae,[145] and 50 Russian beetle species.[146]

Apparently the related order Strepsiptera is still cytologically unknown.

F. HYMENOPTERA

All the Hymenoptera except the few species that are thelytokous have haploid males, produced from unfertilized eggs. The origin of haplodiploidy in this group presumably therefore dates back to its inception in the early Mesozoic or late Palaeozoic. There seems little doubt that this form of sex determination has been the key factor enabling the development of eusociality in the higher groups of the order.[147]

Possible genetic mechanisms underlying haplodiploid sex determination have already been outlined. At least two different models are necessary to fit the observed facts, one involving multiple alleles at a single locus, and the other involving multiple loci.[21,148,149] The single-locus mechanism (see p. 64) results in up to 50% of the fertilized eggs in inbred populations developing as diploid males, which generally have low viability and fertility.[21,150] Diploid males have been reported from several species of Tenthredinoidea, Ichneumonoidea, Apoidea, and Formicoidea.[150] Not all the reported instances can be attributed to inbreeding, but the single-locus model seems to be established for one or more species in each of the above-cited subfamilies, suggesting that it is ancestral to the Hymenoptera as a whole; e.g., the sawfly (*Neodiprion nigroscutum*, the braconid *Habrobracon hebetor*, the ichneumonid *Diadromus pulchellus*,[151] the honeybee *Apis mellifera*, and the fire ant *Solenopsis invicta*.[152]

If single-locus sex determination occurs generally in the ichneumonid and braconid parasitoids reared and released as biological control agents, they may suffer from reduced viability if inbred populations are used, because of diploid male production.[150] In several species of Chalcidoidea, however, in which sibmating is common in nature, inbreeding has not led to the male-biased sex ratios that would be expected if diploid males were being produced, and a multiple-locus model seems to be necessary. A single-locus scheme also does not seem to explain sex determination in six species of meliponine bees, which did not produce diploid males when sibmated,[153] although diploid males were later obtained in another meliponine

species.[154] Neither single-locus nor multiple-locus models seem applicable to the bethyloid *Goniozus nephantidis*, which typically has within-brood mating and hence marked inbreeding.[155] Generalizations would be unwise in the present state of knowledge, but it seems that single-locus sex determination is likely to occur in Hymenoptera that generally practice outbreeding, or in the higher social groups where the production of diploid males can be controlled; for example, diploid male honeybee larvae are eaten by workers about 72 h after eclosion.[156]

There have been estimates of the number of sex-determining alleles for several species, either by crossing different lines (9 alleles, in *H. hebetor*), or by a statistical calculation based on the incidence of diploid males in natural populations (99–19 in *A. mellifera*, depending on population size;[157] 20 in *Melipona compressipes fasciculata*;[154] 15 in *S. invicta*[152]).

The pteromalid (chalcidoid) wasp *Nasonia vitripennis* has on occasions produced fully fertile diploid males in laboratory cultures, but will not do so in response to intensive inbreeding.[158] It is difficult to explain sex determination in this species, even as a multiple-locus mechanism.[10] *Nasonia* has been studied particularly with regard to the ability of the female wasp to manipulate sex ratios by controlling sperm access to eggs, and in the course of those studies several apparently extrachromosomal factors were discovered that influence sex. One of these is of particular interest because it is transmitted paternally, but then inactivates the paternal chromosome set by heterochromatinization in the fertilized egg, so that genomically haploid, all-male broods are obtained.[159] It thus mimics the normal mode of sex determination of some scale insects and of sciarid flies. The transmitting agent has now been identified as an accessory (B) chromosome, termed the paternal sex ratio or PSR chromosome.[160,161] In effect, the PSR chromosome "jumps" from one haploid set to another at the expense of the chromosomes with which it is associated and is thus an extreme example of "selfish" DNA.

Most gall-forming Cynipoidea have two generations per year, one thelytokous and the other bisexual. For several common species it has been shown that females of the unisexual, thelytokous generations differ in the eggs that they lay, producing either only haploid (male) eggs or only diploid (female) eggs.[162] The females of the bisexual generation are also of two types, one giving rise only to the male producers of the next unisexual generation, the other only to the female producers. Thus each female of the bisexual generation has grandchildren of only one sex. Possible underlying genetic mechanisms were discussed by Crozier.[21]

G. THE PANORPOID ORDERS

It is generally agreed that the remaining insect orders — including Lepidoptera and Diptera — form a monophyletic group, with the Mecoptera close to its main stem. It is therefore of interest that, whereas only a minority of species in the higher panorpoid orders have an XX/XO system, XO males are found in all species of Mecoptera so far examined except one (which has a clearly derived multiple sex chromosome system, with X_1X_2Y males).[9] It seems likely, therefore, that the variety of sex chromosome systems found in the remaining orders were all derived from an XX/XO system.

Very little is known about sex determination in the highly specialized order Siphonaptera (fleas), which is placed somewhat uncertainly in the panorpoid complex. Of the four species examined, two were probably XX/XY, and two apparently had multiple sex chromosomes;[9,163] males of one of these latter were X_1X_2Y, and of the other possibly $X_1Y_1X_2Y_2$.

At some stage in one of the two main branches of panorpoid evolution — that leading to the Trichoptera and Lepidoptera — the XX/XO system was replaced by a system involving female heterogamety (XY/XX or ZW/ZZ). Suomalainen[164] discussed the similarity between trichopteran and lepidopteran sex chromosome systems. Both Trichoptera and Lepidoptera have numerous small holocentric chromosomes, with sex chromosomes that are hardly distinguishable in either mitotic or meiotic cell divisions, so much of the early work on sex determination in *Lymantria dispar*[6] and *Bombyx mori*[165] was done without any cytological

information. Robinson's[166] list of chromosome numbers of over 1000 species of Lepidoptera has no information on sex chromosomes. However, Smith's[167] discovery that all or part of the unpaired Y (or W) chromosome in females is heterochromatic in interphase nuclei — and hence that nuclei of female Lepidoptera with XY sex chromosome constitution contain a dense "sex chromatin body" that is not found in male cells — provided a simple method for determining the sex chromosome system. Traut and Mosbacher[168] and Ennis[169] between them found a sex chromatin body in cell nuclei of females of 151 out of a total of 185 species studied. Where females of a species had no sex chromatin body, female somatic or oogonial cells of that species generally had an odd number of chromosomes, one less than in the male, indicating an XO/XX (or ZO/ZZ) sex chromosome system. XO females are more common in some families (e.g., Lasiocampidae, Yponomeutidae, Noctuidae) than others. Genera in several families contain both XY and XO species.[169] A very few species have one or two heterochromatic bodies in male as well as female nuclei, presumably due to presence of constitutive heterochromatin on other chromosomes apart from the Y.

Multiple sex chromosomes (females with XY_1Y_2) have been found in two species of Pyraloidea and two of Tortricoidea.[170] The two Y chromosomes, probably arising by simple dissociation, both associate with the X in the first meiotic division of oocytes to form a trivalent. In female somatic cell nuclei of three of the four species, it was possible to observe two sex chromatin bodies, corresponding to Y_1 and Y_2.

Some species of *Solenobia* (Psychidae) have thelytokous races, and a special mechanism is necessary to ensure that progeny are all heterogametic like their mother. In *S. triquetrella*, which has both diploid and tetraploid thelytokous races, the nucleus that develops as an embryo is derived by fusion of two of the four nuclei resulting from meiosis; as this fusion is always between nonsister nuclei, the sex chromosome heterozygosity is preserved.[171]

Even though very few species have been studied in any detail, it is clear that the genetic mechanisms involved in sex determination in Lepidoptera must be almost as variable as they are in Diptera (see below). In the silkworm *B. mori*, it has long been known that the Y chromosome of the female carries a dominant female-determining gene.[165] Diploid, triploid and tetraploid individuals are female as long as there is at least one Y chromosome, even when the X:Y ratio is 3:1. Intersexes do not occur. Thus any male-determining factors on the X or on autosomes must be very weak in their effect. On the other hand, there is abundant evidence from Goldschmidt's work that sex determination in *L. dispar* depends on a delicate and evolutionarily labile balance between the strengths of a male-determining factor or factors on the X chromosomes and one or more female-determining factors, probably on the Y (reviewed by White;[9] but see also Clarke and Ford[172]).

In several lepidopteran families, including some Lasiocampidae[169] and Saturnidae,[169,173] in the same superfamily as *Bombyx*, the occurrence of XO/XX sex chromosome systems seems to rule out any mechanism based on a dominant Y-borne sex factor and, where closely-related XO and XY species occur, it seems likely that the Y has little or no role in sex determination. In *Ephestia*, at least half the Y chromosome can be lost without any effect on sex determination[174] (although the female-determining factor could still be located on the remaining half). It seems possible, therefore, that the vital and dominant role of the silkworm Y chromosome is somewhat unusual.

The other main branch of the panorpoid complex — that leading to the higher Diptera — also seems to have undergone major changes in the mechanism of sex determination early in its evolution. The most primitive group of Diptera-Nematocera, the Tipuloidea, includes species with XY males, such as *Pales* (=*Nephrotoma*) *ferruginea*, in which the X and Y behave in an almost identical fashion to those of Neuroptera-Plannipennia; i.e., they do not form a bivalent in the first division of meiosis, and take up positions in opposite halves of the spindle ("distance pairing"), before segregating to the poles.[175] Even in Tipuloidea, however, there is evidence of a change in the sex determination mechanism which forms the basis for the variety of systems in the Diptera as a whole. In *P. ferruginea*, XXY individuals are male,[176]

indicating that sex determination is based on a dominant-Y mechanism, which is unlike all the other insect groups covered so far.

The X and Y in most tipulids are very small, and in *Tipula caesia* and *T. pruinosa* they have "disappeared"; it seems probable that the sex chromosomes in these two species, or at least the Y chromosome, have fused with members of a pair of autosomes, so that one of the three pairs of autosomes now bears the sex-determining locus.[9] A similar change seems to have occurred in the two species of the tipulid subfamily Limnobidae (=Limoniinae) studied by Wolf:[177] *Dicranomiya* (=*Limonia*) *trinotata* and *Thaumastoptera calceata*. A species of *Liriope* in the family Ptychopteridae, which is placed phylogenetically somewhere between Tipulidae and Psychodidae, provides support for this idea;[178] it has heteromorphic sex chromosomes (X and Y), but they have "acquired" homologous regions so that they pair to form a bivalent in meiosis, suggesting that they are a neo-X and neo-Y formed by translocation or fusion with a pair of autosomes. In this case, the size of the original Y, or the size differential between the original X and Y, was presumably large enough to ensure that the neo-X and neo-Y are recognizably heteromorphic.

In the Psychodoidea, which appear to be a branch of the dipteran phylogeny arising between the Tipuloidea and Culicoidea, only a few species of sand flies (Psychodidae) have been studied cytologically,[179-181] but these provide a similar picture to the Tipuloidea. Most species have 2n = 6 or 2n = 8 without recognizable sex chromosomes, but one (*Phlebotomus perniciosus*) had 2n = 10 including a small heteromorphic pair of sex chromosomes[179] — presumably the more primitive condition.

In Culicoidea, where many more species have been studied cytogenetically, only the Chaoboridae and the culicid subfamily Anophelinae have heteromorphic sex chromosomes.[182,183] The Chaoboridae and one anopheline species (*Chagasia bathana*) have 2n = 8, with acrocentric X and Y, whereas other Anophelinae and all other Culicidae studied have 2n = 6, perhaps as a result of sex chromosome-autosome fusion. X and Y chromosomes in most mosquito species can, however, be distinguished by their different patterns of staining with Giemsa or quinacrine ("G, C, or Q bands"; e.g., Newton et al.[184]), and slight intraspecific or interspecific differences size can often be attributed to different-sized blocks of constitutive heterochromatin (repetitive, noncoding DNA) (e.g., Mezzanotte et al.[185]). *Anopheles* X and Y chromosomes have extensive heterochromatic regions.[183]

The dominant, male-determining locus (M) of *Aedes aegypti* is on one member of the shortest chromosome pair near the centromere,[186] and has been similarly located in several species of *Culex*,[183] but in one strain of *C. tritaeniorhynchus* in Japan, "M" is on one of the longest chromosome pair.[187] Thus, the sex determinant can alter its position in the genome, a phenomenon which comes into its own in the next superfamily, Chironomoidea.

Members of the other family usually included in Culicoidea, the Dixidae, lack heteromorphic sex chromosomes,[188] as in the Chironomoidea.

Sex determination in Chironomoidea — or at least, in Simuliidae and Chironomidae, since the Ceratopogonidae are little studied — is characterized by two features: (1) there are almost always three chromosome pairs, none of which are heteromorphic; and (2) there is usually a dominant male-determining factor that can apparently occur almost anywhere in the genome and often differs in location between closely related species (Figure 12). In the *Eusimulium vernum* complex alone, for example, five out of the six chromosome arms are involved in sex determination in different species and sibling species.[189] In the *E. aureum* species group, which is unusual in having 2n = 4, either of the two chromosome pairs may serve as the sex chromosomes, and sex factors may occur in any of the four chromosome arms.[190]

Sometimes the location of the sex factor varies within species, in which case sex determination operates as a multiple factor system. For example, the Australian *Chironomus oppositus* species complex includes one form, *whitei*, which is apparently polymorphic for four different sex factor locations, with up to three locations occurring in any one population.[191] Many other examples are now available which support the idea that the sex-determining locus in

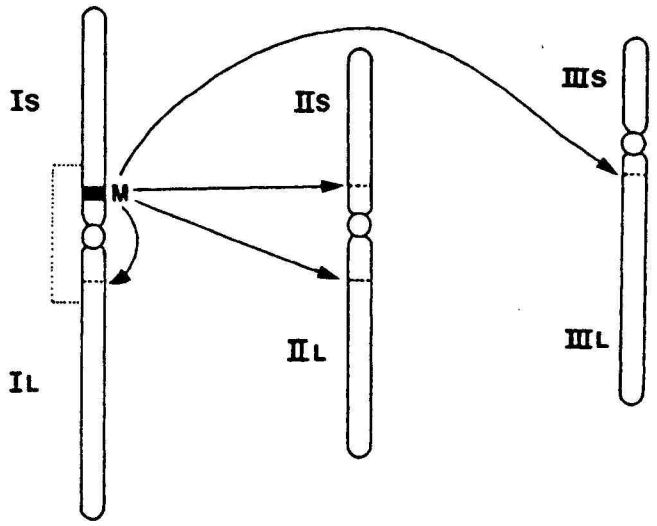

FIGURE 12. Diagrammatic illustration of the mobility of the male-determining factor in blackflies (Simuliidae). The diagram also shows the standard notation used by blackfly cytogeneticists for the six arms of the three chromosome pairs (I, II, and III) of the normal blackfly chromosome complement. Any of these six arms can function as the sex chromosomes, due to transposition of the male-determining factor (M) between chromosomes. The location of M could also be switched from one arm to the other of the same chromosome by a pericentric inversion; for example, an inversion of the section bracketed by a dotted line on chromosome I (although paracentric inversions — those not involving the centromere — are much more common in blackflies).

Chironomoidea — and in many of the higher Diptera discussed below — acts as, or is associated with, a transposable element, and can thus be excised and moved to multiple locations in the genome.[20]

The location of the male sex factor can sometimes be detected cytologically in polytene chromosomes by minor differences in the banding pattern, or by sex-linked inversions. Inversions arise when sections of the chromosome of varying length are excised and then reinserted in the chromosome the "wrong way around". Individuals heterozygous for an inversion can be detected by examining the sequence of bands in the polytene chromosomes, which is inverted in a section of one chromosome in comparison with its homologue. If an inversion is close to or encompasses the sex factor locus, as seems to happen very frequently in blackflies, then it will be partially or completely sex-linked.[192] The frequency of sex-linked inversions also fits the idea of a transposable element being involved in sex determination, because the breakpoints for inversions can also be the sites of excision or insertion of transposable elements.[193]

Rothfels[192] considered that the ancestral condition for Simuliidae and related groups was a complete absence of differentiation of the chromosome carrying the male-determining factor (the notation used for undifferentiated, homomorphic sex chromosomes by simuliid cytogeneticists is X_0Y_0). While this may well be true, it is also possible for the X_0Y_0 condition to be secondary; if, for example, a sex locus associated with an inversion is transposed out of the inversion to a new genomic site, so that the inversion is no longer sex-linked.[194,195]

In *Chironomus tentans,* males are normally heterozygous for a dominant male sex factor, but one population was found that seemed to have female heterogamety; this was interpreted, on the basis of crosses between populations, as due to a dominant female sex factor.[10,196] Nöthiger and Steinmann-Zwicky[11] postulated that this situation might arise by a null mutation of the key gene *Sxl*, accompanied by loss of the dominant male sex factor (M). It has also been suggested, however, that a model involving a weakened male determiner could provide a better explanation of the published results.[197,198]

The remaining groups of Nematocera all have an achiasmate male meiosis, a feature that links them cytogenetically with the higher Diptera. The sex chromosomes of Thaumaleidae and Bibionoidea are usually small, do not form a bivalent, and show "distance pairing" in the first meiotic division, as in tipulids.[176] The Mycetophilidae also have XY males, but the related

families Sciaridae and Cecidomyidae have developed remarkably aberrant chromosome systems, with more chromosomes in the germ line than in the soma (reviewed in detail by White[9]). Neither of these families have Y chromosomes, so that the genic balance between X chromosomes and autosomal factors must be the basis for sex determination.

In *Sciara*, male somatic cells are XO, but the germ line is XX and, after passing through a highly peculiar spermatogenesis, the sperm are homogametic and all carry *two* X chromosomes. Oocytes are normal, with a single X, so all zygotes have *three* X chromosomes, with the potential to develop as either sex. Either one or two of the three X chromosomes are eliminated from presumptive somatic cells at the seventh or eighth cleavage division, to determine the soma of the embryo as either female (XX) or male (XO), respectively. (Germline cells later lose one X chromosome, irrespective of the sex of the embryo, so that they are XX in both sexes.) The sex of the offspring — i.e., whether one or two X chromosomes are eliminated from somatic cells — depends entirely on the genetic constitution of the mother. Certain species of *Sciara* are monogenous, i.e., they invariably have unisexual progenies, so that there are two kinds of mother, male producing and female producing. The latter are thought to be heterozygous for a dominant factor (F), presumably acting through the cytoplasm of the egg to cause the soma of the embryo to develop as female. The ratio of male- to female-producing mothers in such species, and hence the resulting sex ratio, is approximately 1:1. Thus, sex is inherited genetically, but the inheritance is displaced back to the maternal generation. The genetic mechanism could be a null mutation of the *daughterless* gene or its equivalent, as discussed for *Chrysomya* below. Other species of *Sciara* have females that normally produce progeny of both sexes, however, and sex determination in other genera of Sciaridae has still hardly been studied, so it would be unwise to generalize. Haig[199] reviewed the chromosome system of *Sciara coprophila* and developed a model for its evolution based on sex ratio theory.

Cecidomyidae have even more aberrant chromosome systems, with numerous extra ("E") chromosomes in the germ line that are eliminated from somatic cells in early cleavage divisions.[9] In most species studied, there are six chromosomes in male somatic cells and eight in female somatic cells, so that the sex chromosome system is $X_1X_1X_2X_2/X_1X_2O$. This is the case in the hessian fly, *Mayetiola destructor*,[200,201] despite early reports of eight chromosomes in the somatic cells of both sexes. As in *Sciara*, sex is determined by a maternal factor rather than by male heterogamety. Males although X_1X_2O are homogametic, producing only X_1X_2 sperm, so that zygotes are all $X_1X_1X_2X_2$. In male embryos, two X chromosomes (one X_1 and one X_2) are eliminated from presumptive somatic cells at a separate, later cleavage division than that at which the E chromosomes are eliminated; e.g., in *Wachtliella persicariae*, E chromosomes are eliminated at the fourth cleavage division and X chromosomes at the seventh.[202]

As in Sciaridae, many cecidomyids have unisexual progenies,[200,203] but others have the same mothers producing both male and female progeny, and the system by which sex is controlled is unclear. *Heteropeza pygmaea* is best studied in this respect, but Heteropezinae differ from other cecidomyids in that the male somatic cells appear to be haploid, with five chromosomes, whereas female somatic cells have ten chromosomes. However, this only applies when the progeny are produced pedogenetically; in *H. pygmaea*, females reproducing as adults lay only female-determined eggs, but these have five chromosomes as in pedogenetically produced male eggs.[204] Sex determination therefore cannot be based on haplodiploidy, and does not seem to have a genetic basis at all. Went and Camenzind[205] cultured larval ovaries of *H. pygmaea in vitro*, using as culture medium the hemolymph of larvae that had been previously kept in different nutritional environments, and were able to show that the sex of the progeny was dependent on the nutritional conditions experienced by the mother during development.

The more primitive groups of Brachycera have received very little attention from cytogeneticists. In the Tabanoidea, Rhagionidae and Stratiomyidae have XY males where these have

been studied,[206] whereas in Asiloidea, the asilid *Dasyllis* (=*Laphria*) *grossa* is reported to have an XX/XO system.[207] In the more advanced groups of Brachycera (=Cyclorrhapha), there has been detailed work on sex determination mechanisms of representatives of five families: Phoridae (*Megaselia scalaris*), Muscidae (*Musca domestica*), Calliphoridae (*Chrysomya rufifacies, Lucilia cuprina*), Tephritidae (*Ceratitis capitata*), and, of course, Drosophilidae (*D. melanogaster, D. miranda*). These five families span four superfamilies of Cyclorrhapha, so may be fairly representative of the range of mechanisms in the higher Diptera as a whole.

In the phorid fly *M. scalaris*, X and Y chromosomes are not morphologically differentiated, and the male-determining factor (M) is capable of being located on any of the three chromosome pairs,[208] much as in chironomids. In laboratory strains the chromosome (Y) carrying the M factor could be distinguished from its homologue (X) using a combination of cytogenetic and molecular techniques.[209] The segment of the Y chromosome carrying M was found to be conserved in comparison with the homologous region of X, when two unrelated strains were compared. Nevertheless, when the two strains were crossed, four cases were found where the M factor had moved to a different chromosome. The frequency of this change was about 0.06%, which is comparable with known rates of movement of transposable elements in other organisms.[210] The conservation of the M-containing chromosomal region observed in pure strains perhaps indicates that a specific location is favored under certain circumstances, and this could be the first step in the differentiation of new heteromorphic sex chromosomes.

The housefly *M. domestica* provides some particularly interesting examples of evolution of sex-determining systems in progress. It was fully reviewed by Bull,[10] but since then there have been further interesting developments. Earlier European work established that sex determination in houseflies was XX/XY, with heteromorphic sex chromosomes and a presumably dominant male sex factor on the Y. Apart from the Y-borne sex factor, X and Y chromosomes seem to have few or no functional coding regions and are heterochromatic. In strains of non-European origin, however, various sex factors have been found on the autosomes, especially a male determiner (M) near the centromere on autosome 3, and a female determiner (F) on autosome 4 which is epistatic to (i.e., overrides) any number of male-determining factors.[211] In continental Europe, samples from Denmark to Sicily taken in 1975–1981 showed a latitudinal cline: north European populations were all XX/XY, whereas in south and central Italy all populations were XX/XX with sex determined autosomally, the X being totally neutral with regard to sex determination. In southern France, Yugoslavia, and northern Italy, intermediate, mixed populations occurred with all combinations of X and Y in either sex.[212] A very similar north-south cline was found in Japan.[213] The changes in sex determination mechanisms in both southern Europe and Japan are believed to be recent. Various models (e.g., Jayakar[214]) have been advanced to explain this phenomenon; possibly climatic influences are involved, or perhaps the driving force is selective insecticide pressure, as there is now good evidence that pyrethroid/DDT resistance (the "knockdown factor," *Kdr*) is genetically linked with the male-determining locus on autosome 3.[215] However, recent changes have also occurred in the sex-determination system of housefly populations in southeast England, involving an apparent increase in frequency of a male factor on the X chromosome,[216,217] and there is no evidence that loci associated with insecticide resistance (or, in fact, any other functional genes) occur on the housefly X chromosome. Or has the resistance-conferring gene also been transposed to the X from autosome 3, along with the male sex factor? This seems quite possible, since a laboratory housefly strain in Australia was shown to have DDT resistance linked to a male sex factor, but in this case on autosome 2.[218]

Few other Muscidae have been studied cytogenetically. Recent work on *Hydrotaea meriodionalis* indicates a similar story to *M. domestica*, with a dominant autosomal male determiner in some populations and others with XY males.[219] In the closely related Anthomyiidae, the cabbage root fly, *Delia radica* (=*Hylemyia brassicae*), is now known to have a male-determining factor on an autosome (chromosome 6), whereas *D. antiqua* has a small heteromorphic X and Y.[220]

Sex determination in houseflies, with male determiners at various locations in the genome, and the presence of a dominant female determiner in some populations, seems a very different mechanism to the *Drosophila* system, based on an X:A ratio, but there are ways of deriving one from the other fairly simply. Nöthiger and Steinmann-Zwicky[11] suggested, for example, that the dominant female determiner (F) could be a mutation of the key gene *Sxl* to an irrepressible condition, so that it cannot be turned off by M. A similar conclusion was reached by Inoue and Hiroyishi;[221] their model for housefly sex determination incorporates the discovery of a mutation *tra*, closely linked with F on autosome 4; when this is present in the mother, it causes progeny to develop as males even in the absence of any M factors.

In fact, a genic balance/recessive-X system does operate in another muscid, the tsetse fly *G. palpalis*, which also resembles *Drosophila* in that the Y chromosome carries some loci that are necessary for sperm viability, but is not involved in sex determination.[16]

Blowflies (Calliphoridae) generally have small, heteromorphic, and mainly heterochromatic, sex chromosomes. The Y chromosome in *L. cuprina* carries a dominant male sex factor, located near its centromere.[222,223] In *Calliphora erythrocephala*, however, the small heterochromatic pair are no longer sex chromosomes, and the male-determining locus is on one of the other chromosomes, where it is recognizable as a small heterozygosity of the chromomere pattern of the polytene chromosome.[224] And in the monogenic blowfly *C. rufifacies*, sex is controlled by a dominant female determining factor (*F'*) in the mother, *F'f* mothers producing only daughters and *ff* mothers producing only sons, which are therefore also of *ff* genotype. Ullerich,[225] in some elegant experimental work, transplanted pole cells (primordial germ cells) between female embryos of *F'f* and *ff* genotypes. The resultant mothers were germ-line mosaics for *F'f* and *ff*, and both the donor and recipient genotypes were expressed, resulting in a mixture of male and female progeny. Thus, the *F'* gene product is synthesized by the germ-line cells themselves, rather than by maternal somatic cells. Ullerich also did pole cell transplantations between male and female embryos. These resulted in germ-line mosaics that were completely fertile and heterosexual; the donor cells underwent sex reversal and developed as male or female according to their mother's genotype and irrespective of their own genotype. Thus, a genotypically male germ cell can develop as a functional oocyte in a female host, a genotypically female germ cell can develop as a functional sperm in a male host, and sex is determined solely by regulatory factors provided by maternal somatic cells.

Nöthiger and Steinmann-Zwicky[11] postulated that *F'* in *Chrysomya* is similar or identical to the *daughterless* gene (*da*) of *Drosophila*, which is necessary in the mother in order for the key gene *Sxl* to be active (Figure 6). If *f* is the null (mutant) allele da^-, then in homozygous condition it will render *Sxl* of embryonic germ cells inactive, so that all progeny will be sons. DNA sequence homology has now been demonstrated between the *da* gene of *Drosophila* and a polytene band on the *Chrysomya* chromosome that carries the *F'* locus,[226] strongly supporting this hypothesis.

Tephritidae mostly have heteromorphic sex chromosomes (XX/XY), and in several cases a dominant-Y system has been demonstrated, e.g., in the medfly *C. capitata*.[227] X_1X_2Y males occur in some species,[9,228] Indian species in four genera of Trypetinae apparently have homomorphic sex chromosomes,[229] and female heterogamety (XY/XX) has been demonstrated in some Australian species.[230] In *C. capitata*, the sex chromosomes are almost entirely heterochromatic, and the Y chromosome can suffer large deletions without any obvious ill effect; the male-determining factor is located on its long arm close to the centromere.[227] Some repetitive DNA sequences that are specific to or concentrated in the Y chromosome of *C. capitata* were recently isolated.[231]

The sex determination mechanism of *D. melanogaster* was discussed earlier in this chapter, and there are numerous recent reviews.[8,26,232-236] As regards *Drosophila* other than *D. melanogaster*, the most interesting developments have been with *D. miranda*, a species in the *obscura* group which has an X_1X_2Y system, the X_2 and the Y being recently derived (i.e., a neo-X and neo-Y) by translocation to the original Y chromosome of one member of the third

autosome pair found in the closest relatives (*D. pseudobscura, D. persimilis*), leaving its homologue as a neo-X. Chromosome 3 of *D. pseudobscura/persimilis* is also homologous to the right arm of chromosome 2 of *D. melanogaster*. Thus, a very comprehensively mapped chromosome segment has quite recently become a neo-Y, providing considerable scope for study of the degenerative changes that follow from its permanent heterozygosity, and the consequent accumulation of nonfunctional alleles. Comparisons of the neo-Y and its recently homologous neo-X have particularly shown that the neo-Y has acquired inserted DNA sequences that are not present in the neo-X, and appear to represent a novel transposable element that may be involved in the degenerative process.[237-239]

IV. EVOLUTION OF SEX CHROMOSOMES AND SEX DETERMINATION IN INSECTS

It has taken quite a lot of words to provide an outline review of the many and various methods by which sex determination is achieved in the different orders of insects. It is clearly important to distinguish between the sex chromosome systems, which display to the cytogeneticist a remarkable diversity in their form, behavior, and extent of evolutionary change within and between groups, and the underlying molecular mechanisms, which may perhaps show less variation. However, for most insect groups, the molecular genetics of the sex-determining process is still merely a matter of speculation or extrapolation from the paradigm of *Drosophila*.

Much of the discussion on sex chromosome evolution has centered on the Y chromosome. The ideas about the progressive evolutionary degeneration of the Y chromosome discussed earlier in this chapter were developed primarily with regard to vertebrate systems, particularly mammals where the Y chromosome bears a dominant male-determining locus.[19,240,241] The various models that have been proposed[241] all assume a primitive condition where the sex chromosomes are undifferentiated, homologous, and in fact essentially autosomal except at the sex-determining locus, but in time become progressively differentiated as the Y acquires noncoding DNA and the X acquires a system of dosage compensation.[14] Bull[10] reviewed the evidence for progressive sex chromosome differentiation in other groups including insects, and concluded that it could be applied more generally. Nöthiger and Steinmann-Zwicky[11] postulated that the various genetic mechanisms for sex determination in higher Diptera arose by mutations from a primitive state with undifferentiated sex chromosomes and a dominant male determiner, as in some mosquitoes.

However, when insects are looked at as a group, certain qualifications to the model of progressive sex chromosome differentiation and Y degeneration are necessary. First, a model that is applicable to the genic balance systems that seem to predominate in insects has not yet been developed.[14] Second, the XX/XO system predominates in the lower insect orders, and must be the ancestral condition for several major groups, if not for the class Insecta as a whole. Thus, for many insects with XX/XY, if not the majority, the XY condition has arisen by a major chromosomal rearrangement, rather than by a progressive, gradual change from a primitively undifferentiated state. The subsequent evolution of the neo-Y and the homologous region of the neo-X may be comparable in many ways to the process of mammalian sex chromosome differentiation, but the presence from the start of a fully differentiated X chromosome, coupled with the lack of sex determiners on the Y, must have consequences that need to be fully addressed. It would be instructive to compare the molecular changes in the recently acquired neo-XY system of *Drosophila miranda* with the changes following a recent acquisition of "autosomal" sex determination due to the transposition of a male determiner (M) to a new location, e.g., in certain mosquito species or, very recently, in certain populations of *M. domestica*.

Third, although there are examples of loss of homology between a neo-Y and a neo-X, and evidence of accumulation of nonfunctional alleles and repetitive DNA on the Y chromosome,

there is a lack of information about the circumstances which determine whether a neo-XY becomes an evolutionarily stable XY system, or proceeds inevitably towards complete degeneration and eventual loss of the Y chromosome. In the cytologically well-studied Orthoptera, which show much evolutionary change in the sex chromosomes including numerous examples of *de novo* acquisition of XY systems, there are no clear cases where the Y chromosome has been secondarily lost. It seems that a stable condition may sometimes be reached, where it is advantageous to have sex-linked genes retained on the Y chromosome. In Coleoptera, Xy_p systems with a "degenerate Y" are very ancient and show great evolutionary stability. In Heteroptera, secondary loss of Y chromosomes seems to have occurred many times in the course of evolution at the family level, but not between closely related species, indicating that it does not happen fast or frequently.

This leads to the fifth and final point of qualification, which was discussed by Feraday et al.[195] specifically with respect to the evolution of the sex chromosomes of Simuliidae. There has been a tendency to regard sex chromosome differentiation as an inevitable sequence of events, under the influence of mutation and random drift, rather than as an adaptive process. In Simuliidae, any of the three chromosome pairs can be heterozygous for the male-determining sex factor. Usually the only cytological differentiation between the "X" and the "Y" is in the form of inversions, which may be sex-linked but do not form part of any progressive evolutionary sequence of sex chromosome differentiation.[195] White[9] pointed out that if certain autosomal alleles are polymorphic and exert different selective pressures in the two sexes, then it is advantageous to have them linked to the sex chromosomes. In Orthoptera this may be accomplished by centric fusions between sex chromosomes and autosomes to give neo-XY systems.[242] In those Diptera which have single locus, dominant male sex factors, the linkage may be more easily obtained by transposing the sex locus to another position in the genome. Selective advantage is thus important in establishing a new sex chromosome system, and presumably continues to influence the nature and extent of any subsequent differentiation of the X and Y chromosomes.

REFERENCES

1. **Henking, H.,** Untersuchungen über die ersten Entwichlungsvorgange in die Eiern der Insekten. II. Über spermatogénèse und deren Beziehung zur Entwicklung bei *Pyrrhocoris apterus*, *Z. Wiss. Zool. Abt. A.*, 51, 685, 1891.
2. **Bridges, C. B.,** The origin of variations in sexual and sex-limited characters, *Am. Nat.*, 56, 51, 1922.
3. **Bridges, C. B.,** Sex in relation to genes and chromosomes, *Am. Nat.*, 59, 127, 1925.
4. **Bridges, C. B.,** Cytological and genetic basis of sex, in *Sex and Internal Secretions*, 2nd ed., Allen, C., Ed., Williams & Wilkins, Baltimore, 1939, 15.
5. **Goldschmidt, R. B.,** *The Mechanism and Physiology of Sex Determination*, Methuen, London, (translation by W. J. Dakin), 1923.
6. **Goldschmidt, R. B.,** Die Sexuellen Zwischenstufen, *Monogr. Gesamtgeb. Pflanzen Tiere*, Vol. 23, Springer, Berlin, 1931.
7. **Baker, B. S. and Belote, J. M.,** Sex determination and dosage compensation in *Drosophila melanogaster*, *Annu. Rev. Genet.*, 17, 345, 1983.
8. **Baker, B. S.,** Sex in flies: the splice of life, *Nature (London)*, 340, 521, 1989.
9. **White, M. J. D.,** *Animal Cytology and Evolution*, 3rd ed., Cambridge University Press, Cambridge, U.K., 1973.
10. **Bull, J. J.,** *Evolution of Sex-Determining Mechanisms*, Benjamin/Cummings Publishing, Menlo Park, CA, 1983.
11. **Nöthiger, R. and Steinmann-Zwicky, M.,** A single principle for sex determination in insects, *Cold Spring Harbor Symp. Quant. Biol.*, 50, 615, 1985.
12. **Disney, R. H. L. and Cumming, M. S.,** Abolition of Alamirinae and ultimate rejection of Wasmann's theory of hermaphroditism in Termitoxeniinae (Diptera, Phoridae), *Bonn. Zool. Beitr.*, 43, 145, 1992.

13. **Bergerard, J.,** Environmental and physiological control of sex determination and differentiation, *Annu. Rev. Entomol.*, 17, 57, 1972.
14. **Charlesworth, B.,** The evolution of sex chromosomes, *Science*, 251, 1030, 1991.
15. **Ullerich, F.-H.,** Geschlechtschromosomen und Geschlechtsbestimmung bei einigen Calliphoren (Calliphoridae, Diptera), *Chromosoma*, 14, 45, 1963.
16. **Southern, D. I.,** Chromosome diversity in tsetse flies, in *Insect Cytogenetics*, Blackman, R. L., Hewitt, G. M., and Ashburner, M., Eds., Blackwell, Oxford, 1980, 225.
17. **Steinemann, M. and Steinemenn, S.,** Degenerating Y chromosome of *Drosophila miranda*: a trap for retrotransposons, *Proc. Natl. Acad. Sci. U.S.A.*, 89, 7591, 1992.
18. **Rice, W. R.,** Genetic hitchhiking and the evolution of reduced genetic activity of the Y chromosome, *Genetics*, 116, 161, 1987.
19. **Charlesworth, B.,** Model for evolution of Y chromosomes and dosage compensation, *Proc. Natl. Acad. Sci. U.S.A.*, 75, 5618, 1978.

19a. **John, B. and Hewitt, G. M.,** Patterns and pathways of chromosome evolution within the Orthoptera, *Chromosoma*, 25, 40, 1968.

20. **Green, M. M.,** Transposable elements in *Drosophila* and other Diptera, *Annu. Rev. Genet.*, 14, 109, 1980.
21. **Crozier, R. H.,** Hymenoptera, *Animal Cytogenetics* 3, *Insecta* 7, Gebrüder Borntraeger, Berlin/Stuttgart, 1975.
22. **Crozier, R. H.,** Evolutionary genetics of Hymenoptera, *Annu. Rev. Entomol.*, 22, 263, 1977.
23. **Whiting, P. W.,** Multiple alleles in complimentary sex determination of *Habrobracon*, *Genetics*, 28, 365, 1943.
24. **Mackensen, O.,** Viability and sex determination in the honey bee *Apis mellifica*, *Genetics*, 36, 500, 1951.
25. **Smith, S. G. and Wallace, D. R.,** Allelic sex determination in a lower hymenopteran, *Neodiprion nigroscutum* Midd., *Can. J. Genet. Cytol.*, 13, 617, 1971.
26. **Steinmann-Zwicky, M., Amrein, H., and Nöthiger, R.,** Genetic control of sex determination in *Drosophila*, *Adv. Genet.*, 27, 189, 1990.
27. **Chandra, H. S.,** Sex determination: a hypothesis based on noncoding DNA, *Proc. Natl. Acad. Sci. U.S.A.*, 82, 1165, 1985.
28. **Parkhurst, S. M., Bopp, D., and Ish-Horowicz, D.,** X:A ratio, the primary sex-determining signal in *Drosophila*, is transduced by helix-loop-helix proteins, *Cell*, 63, 1179, 1990.
29. **Torres, M. and Sanchez, L.,** The sisterless-b function of the *Drosophila* gene *scute* is restricted to the stage when the X:A ratio determines the activity of *Sex-lethal*, *Development*, 113, 715, 1991.
30. **Keyes, L. N., Cline, T. W., and Schedl, P.,** The primary sex determination signal of *Drosophila* acts at the level of transcription, *Cell*, 68, 933, 1992.
31. **Stewart, B. and Merriam, J.,** Dosage compensation, in *The Genetics and Biology of Drosophila*, Ashburner, M. and Wright, T. R. F., Eds., 2d, Academic Press, London/New York, 1980, 107.
32. **Rao, S. R. V. and Ali, S.,** Insect sex chromosomes 6: a presumptive hyperactivation of the male X chromosome in *Acheta domesticus*, *Chromosoma*, 86, 325, 1982.
33. **Rao, S. R. V. and Arora, P.,** Insect sex chromosomes. III: differential susceptibility of homologous X chromosomes of *Gryllotalpa fossor* to ^{3}H-Urd-induced aberrations, *Chromosoma*, 74, 241, 1979.
34. **Sarkar, S. and Rao, S. R. V.,** Insect sex chromosomes XI. ^{3}H-TdR induces random aberrations in the X chromosomes of *Gryllotalpa fossor* (Orthoptera), *Mutat. Res.*, 282, 113, 1992.
35. **Nagl, W.,** The sex chromatin of *Tachycines asynamorus* (Orthoptera) and its implications, *Cytologia*, 38, 107, 1973.
36. **Johnson, M. S. and Turner, J. R. G.,** Absence of dosage compensation for a sex-linked enzyme in butterflies (*Heliconius*), *Heredity*, 43, 71, 1979.
37. **Nuñez, O.,** Cytology of Collembola, *Nature (London)*, 194, 946, 1962.
38. **Kiauta, B.,** Review of the germ cell chromosome cytology of Collembola, with a list of chromosome numbers and data on two species new to cytology, *Genen Phaenen*, 13, 89, 1970.
39. **Cassagnau, P.,** Les chromosomes polytènes de *Neanura monticola* Cassagnau (Collembola). I. Polymorphisme écologique du chromosome X, *Chromosoma*, 46, 343, 1974.
40. **Charlton, H. H.,** The spermatogenesis of *Lepisma domestica*, *J. Morphol.*, 35, 381, 1921.
41. **Fratello, B.,** Citotassonomia dei Proturi (Insecta, Apterygota), *Atti Congr. Naz. Ital. Entomol. Siena*, 9, 267, 1972.
42. **Fratello, B. and Sabatini, M. A.,** Chromosome studies in Protura Eosentomoidea, in *Third Int. Sem. Apterygota*, Dallai, R., Ed., University of Siena, Italy, 1989, 167.
43. **Mol, A. W. M.,** Notes on the chromosomes of some western European Ephemeroptera, *Chromosome Information Service*, 24, 10, 1978.
44. **Kiauta, B.,** Sex chromosomes and sex-determining mechanisms in Odonata, with a review of the cytological conditions in the family Gomphidae, and references to the karyotype evolution in the order, *Genetica*, 40, 127, 1969.
45. **Kiauta, B.,** Synopsis of the main cytotaxonomic data in the order Odonata, *Odonatologica (Utrecht)*, 1, 73, 1972.

46. **Oksala, T.,** Zytologische Studien an Odonata. I. Chromosomenverhältnisse bei der Gattung *Aeschna* mit besonderer Berücksichtigung der postreduktionellen Teilung der Bivalente, *Ann. Acad. Sci. Fenn. A IV*, 4, 1, 1943.
47. **Tyagi, B. K.,** Cytotaxonomy of the genus *Onychogomphus* Selys (Odonata: Anisoptera, Gomphidae), with special reference to the evolution of the sex-determining mechanism and the reduced chromosome number in the family Gomphidae, in *Proc. 1st Indian Symp. Odonatology*, Mathavan, S., Ed., Madurai Kamaraj University, Madurai, India, 1985, 217.
48. **Matthey, R. and Aubert, J.,** Les chromosomes des Plécoptères, *Bull. Biol. Fr. Belg.*, 81, 202, 1947.
49. **Hewitt, G. M.,** Orthoptera, *Animal Cytogenetics* 3, *Insecta* 1, Gebrüder Borntraeger, Berlin/Stuttgart, 1979.
50. **White, M. J. D.,** Blattodea, Mantodea, Isoptera, Grylloblattodea, Phasmatodea, Dermaptera, Embioptera, *Animal Cytogenetics* 3, *Insecta* 2, Gebrüder Borntraeger, Berlin/Stuttgart, 1976.
51. **Cohen, S. and Roth, L. M.,** Chromosome numbers of the Blattaria, *Ann. Entomol. Soc. Am.*, 63, 1520, 1970.
52. **Luykx, P.,** XO:XX sex chromosomes and Robertsonian variation in the autosomes of the wood-roach *Cryptocercus punctulatus* (Dictyoptera: Blattaria: Cryptocercidae), *Ann. Entomol. Soc. Am.*, 76, 518, 1983.
53. **Luykx, P.,** A cytogenetic survey of 25 species of lower termintes from Australia, *Genome*, 33, 80, 1990.
54. **Tyagi, B. K.,** Review of the cytotaxonomy of Isoptera (Insecta) with a description of the male germ cell chromosomes of *Microcerotermes beesoni* Snyder from the Dehradun Vallet, India, *Indian Rev. Life Sci.*, 7, 263, 1987.
55. **Vincke, P. P. and Tilquin, J. B.,** A sex-linked ring quadrivalent in Termitidae (Isoptera), *Chromosoma*, 67, 151, 1978.
56. **Fontana, F.,** Cytological analysis of the chromosome complement of *Kalotermes flavicollis* Fabr. (Isoptera, Kalotermitidae). The Sex determining mechanism, *Cytologia*, 47, 147, 1982.
57. **Syren, R. M. and Luykx, P.,** Permanent segmental interchange complex in the termite *Incisitermes schwarzi*, *Nature (London)*, 266, 167, 1987.
58. **Syren, R. M. and Lyukx, P.,** Geographic variation of sex-linked translocation heterozygosity in the termite *Kalotermes approximatus* Snyd. (Insecta: Isoptera), *Chromosoma*, 82, 65, 1981.
59. **Syren, R. M. and Luykx, P.,** Experimental hybridization between chromosomal races in *Kalotermes approximatus*, a termite with extensive sex-linked translocation heterozygosity, *Chromosoma*, 83, 563, 1981.
60. **Luykx, P. and Syren, R. M.,** Multiple sex-linked reciprocal translocations in a termite from Jamaica, *Experientia*, 37, 819, 1981.
61. **Luykx, P. and Syren, R. M.,** The cytogenetics of *Incisitermes schwarzi* and other Florida termites, *Sociobiology*, 4, 191, 1979.
62. **White, M. J. D.,** The evolution of sex chromosomes I. The XO and X_1X_2Y mechanisms in praying mantids, *J. Genet.*, 42, 143, 1941.
63. **Callan, H. G. and Jacobs, P. A.,** The meiotic process in *Mantis religiosa* males, *J. Genet.*, 55, 200, 1957.
64. **Liebenberg, H., Fossey, A., and Jacobs, D. H.,** An unexpected sex chromosome mechanism in a South African mantid *Polyspilota aeriginosa* Goez, *Caryologia*, 44, 195, 1991.
65. **Craddock, E.,** Chromosomal evolution and speciation in *Didymuria*, in *Genetic Mechanisms of Speciation in Insects*, White, M. J. D., Ed., Australia and New Zealand Book Co., Sydney, 1974, 24.
66. **Bergérard, J.,** Intersexualité experimentale chez *Carausius morosus* Br. (Phasmidae), *Bull. Biol. Fr. Belg.*, 95, 273, 1961.
67. **Pijnacker, L. P.,** Effect of centrifugation of the eggs on the sex of *Carausius morosus* Br., *Nature (London)*, 210, 1184, 1966.
68. **Pijnacker, L. P.,** Effects of X-rays on different meiotic stages of oocytes in the parthenogenetic stick insect *Carausius morosus* Br., *Mutat. Res.*, 13, 251, 1971.
69. **l'Helias, C. and Boulanger-Sandrin, J.,** Influence des ptérines réduites et de l'hormone juvenile sur l'intersexualité du Phasme *Carausius morosus*, *Ann. Endocrinol.*, 37, 189, 1976.
70. **Pijnacker, L. P. and Ferwerda, M. A.,** Sex chromosomes and origin of males and sex mosaics of the parthenogenetic stick insect *Carausius morosus*, *Chromosoma*, 79, 105, 1980.
71. **Pijnacker, L. P. and Harbott, J.,** Structural heterozygosity and aneuploidy in the parthenogenetic stick insect *Carausius morosus* Br. (Phasmatodea: Phasmatidae), *Chromosoma*, 76, 165, 1980.
72. **Mesa, A., Ferreira, A., and Carbonell, C. S.,** Cariologie de los acridoides neotropicales, estado actual de su conocimiento y nuevas contribucones, *Ann. Soc. Entomol. Fr.*, 18, 507, 1982.
73. **Yadav, J. S. and Yadav, A. S.,** X-autosome fusion in catantopine grasshoppers (Acridoidea: Orthoptera), *Cytobios*, 61, 21, 1990.
74. **Bugrov, A. G.,** [Neo-XY sex chromosome determination in grasshoppers *Asiotmethis heptaopamicus* (Zub.) and *Atrichotmethis semenovi* (Zub.) (Orthoptera: Pamphagidae)], *Tsitologiya*, 28, 117, 1986.
75. **Yadav, J. S. and Yadav, A. S.,** Chromosome number and sex-determining mechanisms in 30 species of Indian Orthoptera, *Folia. Biol. (Krakow)*, 34, 277, 1986.
76. **Fernandez-Piqueras, J., Rojo Garcia, E., and Sentis Castano, C.,** A tandem fusion origin of a neo XY sex determining mechanism in the long-horned *Callicrania seoanei* (Bol.), *Heredity*, 47, 397, 1981.
77. **Fernandez-Piqueras, J., Rodriguez Campos, A., Sentis Castano, C., and Rojo Garcia, E.,** Sex chromosome evolution in the polytypic species *Pycnogaster cucullata* (Charp.), *Heredity*, 50, 217, 1983.

78. **Sentis Castano, C. and Fernandez-Piqueras, J.,** Nature and distribution of heterochromatinized regions in the chromosomal races of *Pycnogaster cucullata* (Insecta, Orthoptera), *Genetica*, 72, 127, 1987.
79. **White, M. J. D., Blackith, R. E., Blackith, R. M., and Cheney, J.,** Cytogenetics of the *viatica* group of morabine grasshoppers: I. The "coastal" species, *Austr. J. Zool.*, 15, 263, 1967.
80. **Hewitt, G. M. and John, B.,** Inter-population sex chromosome polymorphism in the grasshopper *Podisma pedestris*. II. Population parameters, *Chromosoma*, 37, 23, 1972.
81. **Bella, J. L., Westerman, M., Lopez Fernandez, C., Delatorre, J., Rubio, J. M., and Gosalvez, J.,** Sex chromosome and autosome divergence in podisma (Orthoptera) in Western Europe, *Genet. Sel.*, 23, 5, 1991.
82. **Sands, V. E.,** The neo-XY system of *Catantops humilis* (Acrididae: Catantopinae) in Malaysia, *Biol. J. Linn. Soc.*, 39, 269, 1990.
83. **Saltet, P.,** Les Dolichopodes de Corse (Orthoptera — Raphidophoridae) 1° étude cytologique preliminaire, *Bull. Soc. Hist. Nat. Toulouse*, 104, 165, 1967.
84. **Webb, G. C. and White, M. J. D.,** A new interpretation of the sex-determining mechanism of the European earwig, *Forficula auricularia*, *Experientia*, 26, 1387, 1970.
85. **Meinander, M., Halkka, O., and Söderland, V.,** Chromosomal evolution in the Psocoptera, *Not. Entomol.*, 54, 81, 1974.
86. **Buxton, P. A.,** *The Louse*, Edward Arnold, London, 1939.
87. **Ueshima, N.,** Hemiptera. II: Heteroptera, *Animal Cytogenetics* 3, *Insecta* 6, Gebrüder Borntraeger, Berlin/Stuttgart, 1979.
88. **Muramoto, N.,** A chromosomal study of thirty Japanese heteropterans (Heteroptera), *Genetica*, 49, 37, 1978.
89. **Calabrese, D. M. and Tallerico, P.,** Cytogenetic study in males of Nearctic genera of Gerridae (Hemiptera: Heteroptera), *Proc. Entomol. Soc. Wash.*, 86, 354, 1984.
90. **Nokkala, S. and Nokkala, C.,** The occurrence of the XO sex chromosome system in *Dictyonota tricornis* (Schr.) Tingidae Hemiptera and its significance for concepts of sex chromosome system evolution in Heteroptera, *Hereditas*, 100, 299, 1984.
91. **Schuh, R. T.,** The influence of cladistics on heteropteran classification, *Annu. Rev. Entomol.*, 31, 67, 1986.
92. **Cobben, R. H.,** *Evolutionary Trends in Heteroptera. I. Eggs, Architecture of the Shell, Gross Embroylogy and Eclosion*, Center for Agricultural Publication and Documentation, Wageningen, 1968, 374.
93. **Messthaler, H. and Traut, W.,** Phases of sex chromosome inactivation in *Oncopeltus fasciatus* and *Pyrrhocoris apterus* (Insecta, Heteroptera), *Caryologia*, 28, 501, 1975.
94. **Thomas, D. B., Jr.,** Chromosome evolution in the Heteroptera (Hemiptera): Agmatoploidy versus aneuploidy, *Ann. Entomol. Soc. Am.*, 80, 720, 1987.
95. **Emeljanov, A. F. and Kirillova, V. I.,** Trends and modes of karyotype evolution in the Cicadina (Homoptera). I. Karyotypic peculiarities and evolutionary changes in the karyotypes of cicadas of suprafamily Cicadelloidea, *Entomol. Rev. (USSR)*, 69, 62, 1990.
96. **Emeljanov, A. F. and Kirillova, V. I.,** [Trends and types of karyotype evolution in Cicadina (Homoptera). II. Peculiarities and evolutionary changes of the karyotypes in the superfamilies Cercopoidea, Cicadoidea, Fulgoroidea and in the Cicadina as a whole, *Entomol. Rev. (USSR)*, 71, 59, 1992.
97. **den Hollander, J.,** The chromosomes of *Niloparvata lugens* Stal. amd some other Auchenorrhyncha, *Cytologia*, 47, 227, 1982.
98. **John, B. and Claridge, M. F.,** Chromosome variation in British populations of *Oncopsis* (Hemiptera: Cicadellidae), *Chromosoma*, 46, 77, 1974.
99. **Maryańska-Nadachowska, A., Kuznetsova, V. G., and Warchalowska-Sliwa, E.,** Karyotypes of Psyllina (Homoptera). I. New data and check list, *Folia Biol. (Kraków)*, 40, 15, 1992.
100. **Maryańska-Nadachowska, A. and Hodkinson, I. D.,** Karyotypes of Psylloidea. II. Chromosome numbers of nine Mediterranean species from Mallorca (Spain), *Folia Biol. (Kraków)*, 41, 1, 1993.
101. **Maryańska-Nadachowska, A., Glowacka, E., and Warchalowska-Sliwa, E.,** Karyotypes of Psylloidea (Homoptera). III. Chromosome numbers of eight species belonging to the families Aphalaridae, Psyllidae, Homotomidae and Triozidae, *Folia Biol. (Kraków)*, 41, 7, 1993.
102. **Schrader, F.,** Sex determination in the white-fly (*Trialeurodes vaporariorum*), *J. Morphol.*, 34, 267, 1920.
103. **Thomsen, M.,** Studien über die Parthenogenese bei einigen Cocciden und Aleurodiden, *Z. Zellforsch. Mikrosk. Anat.*, 5, 1, 1927.
104. **van Lenteren, J. C. and Noldus, P. J. J.,** Whitefly-plant relationships: behavioural and ecological aspects, in *Whiteflies: Their Bionomics, Pest Status and Management*, Gerling, D., Ed., Intercept, Andover, U.K., 1990, 47.
105. **Sharaf, N. and Batta, Y.,** Effect of some factors on the relationship between the whitefly *Bemisia tabaci* Genn. (Homopt., Aleyrodidae) and the parasitoid *Eretmocerus mundus* Mercet (Hymenopt., Aphelinidae), *Z. Angew. Entomol.*, 99, 267, 1985.
106. **Nur, U.,** Unusual chromosome systems in scale insects, in *Insect Cytogenetics*, Blackman, R. L., Hewitt, G. M., and Ashburner, M., Eds., Blackwell, Oxford, 1980, 97.
107. **Nur, U.,** Chromosomes, sex-ratios and sex determination, in *Armored Scale Insects, Their Biology, Natural Enemies and Control*, Rosen, D., Ed., Elsevier, Amsterdam, 1990, 179.

108. **Brown, S. W.,** Adaptive status and genetic regulation in major evolutionary changes of coccid chromosome systems, *Nucleus*, 20, 145, 1977.
109. **Haig, D.,** The evolution of unusual chromosomal systems in coccoids: extraordinary sex ratios revisited, *J. Evol. Biol.*, 6, 69, 1993.
110. **Blackman, R. L. and Hales, D. F.,** Behaviour of the X chromosomes during growth and maturation of parthenogenetic eggs of *Amphorophora tuberculata* (Homoptera, Aphididae), in relation to sex determination, *Chromosoma*, 94, 59, 1986.
111. **Orlando, E.,** Sex determination in *Megoura viciae* Buckton (Homoptera, Aphididae), *Monit. Zool. Ital. (N.S.)*, 8, 61, 1974.
112. **Blackman, R. L.,** Chromosomes and parthenogenesis in aphids, in *Insect Cytogenetics*, Blackman, R. L., Hewitt, G. M., and Ashburner, M., Eds., Blackwell, Oxford, 1980, 133.
113. **Hales, D. F. and Mittler, T. E.,** Chromosomal sex determination in aphids controlled by juvenile hormone, *Genome*, 29, 107, 1987.
114. **Kawada, K.,** Polymorphism and morph determination, in *Aphids, Their Biology, Natural Enemies and Control*, 2A, Minks, A. K. and Harrewijn, P., Eds., Elsevier, Amsterdam, 1987, 255.
115. **Blackman, R. L.,** Spermatogenesis in the aphid *Amphorophora tuberculata* (Homoptera, Aphididae), *Chromosoma*, 92, 357, 1985.
116. **Blackman, R. L.,** Stability of a multiple X chromosome system and associated B chromosomes in birch aphids (*Euceraphis* spp., Homoptera: Aphididae), *Chromosoma*, 96, 318, 1988.
117. **Blackman, R. L.,** The chromosomes of Lachnidae, *Acta Phytopathol. Entomol. Hung.*, 25, 273, 1990.
118. **Hales, D. F.,** The chromosomes of *Schoutedenia lutea* (Homoptera, Aphidoidea, Greenideinae), with an account of meiosis in the male, *Chromosoma*, 98, 295, 1989.
119. **Blackman, R. L.,** Aphid cytology and genetics, in *Evolution and Biosystematics of Aphids*, Szelegiewicz, H., Ed., Ossolineum, Warsaw, 1985, 171.
120. **Morgan, T. H.,** The predetermination of sex in phylloxerans and aphids, *J. Exp. Zool.*, 19, 285, 1915.
121. **Steffan, A. W.,** Zum Generation- und Chromosomenzyklus der Adelgidae (Homoptera: Aphidina), *Verh. Dtsch. Zool. Ges.*, 1967, 762.
122. **Bournier, A.,** Contribution à l'étude de la parthénogenèse des Thysanoptères et de sa cytologie, *Arch. Zool. Exp. Gen.*, 93, 219, 1956.
123. **Risler, H. and Kempter, E.,** Die Haploidie der Männchen und die Endopolyploidie in einigen Geweben von *Haplothrips* (Thysanoptera), *Chromosoma*, 12, 351, 1961.
124. **Mound, L. A.,** Patterns of sexuality in Thysanoptera, in 1991 Conference on Thrips (Thysanoptera): Insect and Disease Considerations in Sugar Maple Management, U.S.D.A. Forest Service, North Eastern Forest Experimental Station General Tech. Rep. NE-161, University Park, PA, 1991, 2.
125. **Ananthakrishnan, T. N.,** *Bioecology of Thrips*, Indira Publishing House, Oak Park, MI, 1984.
126. **Crespi, B. J.,** Sex-ratio selection in a bivoltine thrips. I. Conditional sex-ratio manipulation and fitness variation, *Evolution*, 42, 1199, 1988.
127. **Hughes-Schrader, S.,** Distance segregation and compound sex chromosomes in mantispids (Neuroptera: Mantispidae), *Chromosoma*, 27, 109, 1969.
128. **Hughes-Schrader, S.,** Segregational mechanisms of sex chromosomes in Megaloptera (Neuropteroidea), *Chromosoma*, 81, 307, 1980.
129. **Hennig, W.,** *Insect Phylogeny* (English translation by Pont, A. C.), Wiley, Chichester, U.K., 1981.
130. **Smith, S. G. and Virkki, N.,** Coleoptera, *Animal Cytogentics* 3, *Insecta* 5, Gebrüder Borntraeger, Berlin/Stuttgart, 1978.
131. **Virkki, N.,** Chromosomes in evolution of Coleoptera, in *Chromosomes in Evolution of Eukaryotic Groups*, Vol. 2, Sharma, A. K. and Sharma, A., Eds., CRC Press, Boca Raton, FL, 1984, 41.
132. **Virkki, N., Mazzella, C., and Denton, A.,** Silver staining of the coleopteran Xy_p sex bivalent, *Cytobios*, 67, 45, 1991.
133. **Postiglioni, A., da Silva, A., de Leon, R., and de Vaio, E. S.,** Three species of *Helipodus* (Coleoptera, Curculionidae) with different karyotypes and sex chromosome systems, *Genetica*, 75, 213, 1987.
134. **Serrano, J. and Yadav, J. S.,** Chromosome numbers and sex-determining mechanisms in adephagan Coleoptra, *Coleopt. Bull.*, 38, 335, 1984.
135. **Maddison, D. R.,** Chromosomal diversity and evolution in the ground beetle genus *Bembidion* and related taxa (Coleoptera: Carabidae: Trechitae), *Genetica*, 66, 93, 1985.
136. **Yadav, J. S., Kondal, K., and Yadav, A. S.,** Cytology of *Cicindela (Myriochile) undulata* and *C. (M.) fastidiosa* with a summary of chromosomal data on the Cicindelidae, *Cicindela*, 17, 1, 1985.
137. **Mesa, A. and Fontanetti, C. S.,** The chromosomes of a primitive species of beetle, *Ytu zeus* (Coleoptera, Myxophaga, Torridincolidae), *Proc. Acad. Nat. Sci. Philadelphia*, 137, 102, 1985.
138. **Petitpierre, E. and Segarra, C.,** Chromosomal variability and evolution of Chrysomelidae (Coleoptera), particularly that of Chrysomelinae and palaearctic Alticinae, *Entomography*, 3, 403, 1985.
139. **Petitpierre, E.,** Recent advances in the evolutionary cytogenetics of the leaf beetles (Coleoptera, Chrysomelidae), *Entomography*, 6, 433, 1989.

140. **Yadav, J. S. and Dange, M. P.,** Chromosomal investigations on eight species of histerids (Coleoptera: Histeridae), *Elytron (Barcelona)*, 3, 103, 1989.
141. **Yadav, J. S. and Dange, M. P.,** Chromosome number and sex determining mechanisms in twenty species of Indian rove beetles (Staphylinidae: Polyphaga), *Cell Chromosome Res.*, 10, 23, 1987.
142. **Gill, T. K., Gulati, M., and Pajni, H. R.,** Chromosome numbers in Indian weevils (Coleoptera: Curculionidae), *Coleopt. Bull.*, 44, 437, 1990.
143. **Yadav, J. S., Burra, M. R., and Dange, M. P.,** Chromosome number and sex determining mechanism in 32 species of Indian Coleoptera (Insecta), *Nat. Acad. Sci. Lett. (India)*, 12, 93, 1989.
144. **Juan, C. and Petitpierre, E.,** Chromosome numbers and sex-determining systems in Tenebrionidae (Coleoptera), in *Advances in Coleopterology*, Zunino, M., Belles, X., and Blas, M., Eds., European Association of Coleopterology, Barcelona, 1992.
145. **Handa, S. M. and Kochhar, N.,** Cytology of bruchids: I. Chromosome number and sex mechanism of seven cytologically unknown species of bruchids, *Res. Bull. Panjab Univ. Sci.*, 37, 145, 1987.
146. **Yadav, J. S., Lyapunova, E. A., and Vorontsov, N. N.,** Chromosome numbers and sex-chromosome mechanisms in fifty species of Coleoptera from USSR, *Folia Biol. (Krakow)*, 34, 269, 1986.
147. **Trivers, R. L. and Hare, H.,** Haplodiploidy and the evolution of social insects, *Science*, 191, 249, 1976.
148. **Luck, R. F., Stouthamer, R., and Nunney, L.,** Sex determination and sex ratio patterns in parasitic Hymenoptera, in *Evolution and Diversity of Sex Ratio in Haplodiploid Insects and Mites*, Wrensch, D. L. and Ebbert, M. A., Eds., Chapman and Hall, Engelwood Cliffs, NJ, 1993.
149. **Cook, J. M.,** Experimental tests of sex determination in *Goniozus nephantidis* (Hymenoptera: Bethylidae), *Heredity*, 71, 130, 1993.
150. **Stouthamer, R., Luck, R. F., and Werren, J. H.,** Genetics of sex determination and the improvement of biological control using parasitoids, *Environ. Entomol.*, 21, 427, 1992.
151. **Periquet, T. G., Hedderwick, M. P., El Agoze, M., and Poirie, M.,** Sex determination in the hymenopteran *Diadromus pulchellus* (Ichneumonidae): validation of the one-locus multi-allele model, *Heredity*, 70, 420, 1993.
152. **Ross, K. G. and Fletcher, D. J. C.,** Genetic origin of male diploidy in the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae), and its evolutionary significance, *Evolution*, 39, 888, 1985.
153. **Kerr, W. E. and Nielsen, R. A.,** Sex determination in bees (Apinae), *J. Apic. Res.*, 6, 3, 1967.
154. **Kerr, W. E.,** Sex determination in bees. XXI. Number of XO-heteroalleles in a natural population of *Melipona compressipes fasciculata* (Apidae), *Insectes Soc.*, 34, 274, 1987.
155. **Cook, J. M.,** Sex determination in the Hymenoptera: a review of models and evidence, *Heredity*, 71, 421, 1993.
156. **Woyke, J.,** Sex determination, in *Bee Genetics and Breeding*, 1st edition, Rinderer, T. E. (Ed.), Academic Press, Orlando, FL, 1986, 91.
157. **Adams, J., Rothman, E. D., Kerr, W. E., and Paulino-Simões, Z. L.,** Estimation of the number of sex alleles and queen matings from diploid male frequencies in a population of *Apis mellifera*, *Genetics*, 86, 583, 1977.
158. **Skinner, S. W. and Werren, J. H.,** The genetics of sex determination in *Nasonia vitripennis*, *Genetics*, 94, 598, 1980.
159. **Werren, J. H., Nur, U., and Eickbush, D.,** An extrachromosomal factor causing loss of paternal chromosomes, *Nature (London)*, 327, 75, 1987.
160. **Werren, J. H.,** The paternal sex-ratio chromosome of *Nasonia*, *Am. Nat.*, 137, 392, 1991.
161. **Reed, K. M.,** Cytogenetic analysis of the paternal sex ratio chromosome of *Nasonia vitripennis*, *Genome*, 36, 157, 1993.
162. **Folliot, R.,** Contribution à l'étude de la biologie des cynipides gallicoles (Hyménoptères, Cynipoidea), *Ann. Sci. Nat. Zool. Biol. Anim. Zool. Ser.*, 12(6), 407, 1964.
163. **Bayreuther, K.,** Die Cytogenetik zweier norddeutscher Populationen von *Nosopsyllus fasciatus* Bosc. (Aphaniptera), *Chromosoma*, 27, 20, 1969.
164. **Suomalainen, E.,** Achiasmatische Oogenese bei Trichopteren, *Chromosoma*, 18, 201, 1966.
165. **Tazima, Y.,** *Genetics of the Silkworm*, Logos Press, Bristol, 1964.
166. **Robinson, R.,** *Lepidoptera Genetics*, Pergamon Press, Oxford, 1971.
167. **Smith, S. G.,** Heteropycnosis as a means of diagnosing sex, *J. Hered.*, 36, 194, 1945.
168. **Traut, W. and Mosbacher, G. C.,** Geschlechtschromatin bei Lepidopteren, *Chromosoma*, 25, 343, 1968.
169. **Ennis, T. J.,** Sex chromatin and chromosome numbers in Lepidoptera, *Can. J. Genet. Cytol.*, 18, 119, 1976.
170. **Suomalainen, E.,** On the sex chromosome trivalent in some lepidopteran females, *Chromosoma*, 28, 298, 1969.
171. **Seiler, J. and Schäffer, K.,** Untersuchungen über die Entstehung der Parthenogenese bei *Solenobia triquetrella* F.R. (Lepidoptera, Psychidae). II. Analyse der diploidparthenogenetischen *S. triquetrella*, Verhalten, Aufzuchtresultate und Zytologie, *Chromosoma*, 11, 29, 1960.
172. **Clarke, C. and Ford, E. B.,** Intersexuality in *Lymantria dispar* (L.). A reassessment, *Proc. R. Soc. London, Ser. B*, 206, 381, 1980.

173. **Gupta, M. L. and Narang, R. C.,** Karyotype and meiotic mechanism in muga silkmoths, *Antheraea compta* Roth. and *A. assamensis* (Helf.) (Lepidoptera: Saturnidae), *Genetica*, 57, 21, 1981.
174. **Traut, W., Weith, A., and Traut, G.,** Structural mutants of the W chromosome in *Ephestia* (Insecta, Lepidoptera), *Genetica*, 70, 69, 1986.
175. **Fuge, H.,** The 3-dimensional architecture of chromosome fibres in the cranefly. II. Amphitelic sex univalents in meiotic anaphase I, *Chromosoma*, 91, 322, 1985.
176. **Ullerich, F. H., Bauer, H., and Dietz, R.,** Geschlechtsbestimmung bei Tipuliden (Nematocera; Diptera), *Chromosoma*, 15, 591, 1964.
177. **Wolf, E.,** Die Chromosomen in der Spermatogenese einiger Nematocera, *Chromosoma*, 2, 192, 1941.
178. **White, M. J. D.,** Cytological evidence on the phylogeny and classification of the Diptera, *Evolution*, 3, 252, 1949.
179. **White, G. E. and Killick-Kendrick, R.,** Polytene chromosomes of the sandfly *Lutzomyia longipalpis* and the cytogenetics of Psychodidae in relation to other Diptera, *J. Entomol. Ser. A*, 50, 187, 1975.
180. **Troiano, G.,** Heterozygous heterochromatin in giemsa C-banded chromosomes of *Clogmia albipunctata* (*Telmatoscopus albipunctatus*) (Diptera: Psychodidae), *Caryologia*, 41, 201, 1988.
181. **Kreutzer, R. D., Modi, G. B., Tesh, R. B., and Young, D. G.,** Brain cell karyotypes of six species of New and Old World sand flies (Diptera: Psychodidae), *J. Med. Entomol.*, 24, 609, 1987.
182. **Kitzmiller, J. B.,** Genetics, cytogenetics and evolution of mosquitoes, *Adv. Genet.*, 18, 315, 1976.
183. **White, G. B.,** Academic and applied aspects of mosquito cytogenetics, in *Insect Cytogenetics*, Blackman, R. L., Hewitt, G. M., and Ashburner, M., Eds., Blackwell, Oxford, 1980, 245.
184. **Newton, M. E., Southern, D. I., and Wood, R. J.,** X and Y chromosomes of *Aedes aegypti* (L.) distinguished by Giemsa C-banding, *Chromosoma*, 49, 41, 1974.
185. **Mezzanote, R., Ferrucci, L., and Contini, C.,** Identification of sex chromosomes and characterization of the heterochromatin in *Culiseta longiaerolata* (Macquart 1838), *Genetica*, 50, 135, 1979.
186. **Newton, M. E., Wood, R. J., and Southern, D. I.,** Cytological mapping of the M and D loci in the mosquito, *Aedes aegypti* (L.), *Genetica*, 48, 137, 1978.
187. **Baker, R. H. and Sakai, R. K.,** Male-determining factor on chromosome 3 in the mosquito, *Culex tritaeniorhynchus*, *J. Hered.*, 67, 289, 1976.
188. **Frizzi, G., Contini, C., and Mameli, M.,** Ulteriori recherche citogenetiche sui Dixidae della Sardegna, *Atti Assoc. Genet. Ital.*, 11, 286, 1966.
189. **Brockhouse, C.,** Sibling species and sex chromosomes in *Eusimulium vernum* (Diptera: Simulidae), *Can. J. Zool.*, 63, 2145, 1985.
190. **Leonhardt, K. G. and Feraday, R. M.,** Sex chromosome evolution and population differentiation in the *Eusimulium aureum* group of black flies, *Genome*, 32, 543, 1989.
191. **Martin, J. and Lee, B. T. O.,** A phylogenetic study of sex determiner location in a group of Australasian *Chironomus* species (Diptera, Chironomidae), *Chromosoma*, 90, 190, 1984.
192. **Rothfels, K. H.,** Chromosomal variability and speciation in black flies, in *Insect Cytogenetics*, Blackman, R. L., Hewitt, G. M., and Ashburner, M., Eds., Blackwell, Oxford, 1980, 207.
193. **Smith, P. a. and Corces, V. G.,** *Drosophila* transposable elements: mechanisms of mutagenesis and interactions with the host genome, *Adv. Genet.*, 29, 229, 1991.
194. **Mason, G. F.,** Sex chromosome polymorphism in the *Simulium tuberosum* complex (Lundström) (Diptera: Simulidae), *Can. J. Zool.*, 62, 647, 1984.
195. **Feraday, R. M., Leonhardt, K. G., and Brockhouse, C. L.,** The role of sex chromosomes in black fly evolution, *Genome*, 32, 538, 1989.
196. **Thompson, P. E. and Bowen, J. S.,** Interactions of differential primary sex factors in *Chironomus tentans*, *Genetics*, 70, 491, 1972.
197. **Feraday, R. M.,** Weak male-determining genes and female heterogamety in *Chironomus tentans*, *Can. J. Genet. Cytol.*, 26, 748, 1984.
198. **Martin, J. and Lee, B. T. O.,** Are there female heterogametic strains of *Chironomus tentans* Fabricius?, *Can. J. Genet. Cytol.*, 26, 743, 1984.
199. **Haig, D.,** The evolution of unusual chromosome systems in sciarid flies: intragenomic conflict and the sex ratio, *J. Evol. Biol.*, 6, 249, 1993.
200. **Stuart, J. J. and Hatchett, J. H.,** Cytogenetics of the hessian fly. I. Mitotic karyotype analysis and polytene chromosome correlations, *J. Hered.*, 79, 184, 1988.
201. **Stuart, J. J. and Hatchett, J. H.,** Cytogenetics of the hessian fly. II. Inheritance and behaviour of somatic and germ-line-limited chromosomes, *J. Hered.*, 79, 190, 1988.
202. **Geyer-Duszyn, I.,** Experimental research and chromosome elimination in Cecidomyidae (Diptera), *Chromosoma*, 11, 499, 1959.
203. **Kozlova, L. V.,** [On monogeny of gall midges, *Aphidoletes aphidimyza* Rond. (Diptera: Cecidomyidae)], *Nauchn. Tr. Leningr. Ord. Skh. Inst.*, No. 374, 11, 1979.
204. **Went, D. F. and Camenzind, R.,** Sex determination in the dipteran insect *Heteropeza pygmaea*, *Genetica*, 52/53, 373, 1980.

205. **Went, D. F. and Camenzind, R.,** Haemolymph-dependent sex determination in a paedogenetic gall midge, *Naturwissenschaften*, 64, 276, 1977.
206. **Boyes, J. W.,** The chromosomes of Rhagionidae, Stratiomyidae and Xylomyidae (Diptera), *Can. J. Genet. Cytol.*, 15, 255, 1973.
207. **Metz, C. W.,** Chromosome studies in the Diptera. IV. Incomplete synapsis in *Dasyllis grossa*, *Biol. Bull. (Wood's Hole, Mass.)*, 43, 253, 1922.
208. **Mainz, F.,** The genetics of *Megaselia scalaris* Loew (Phoridae): a new type of sex determination in Diptera, *Am. Nat.*, 98, 415, 1964.
209. **Willhoeft, U. and Traut, W.,** Molecular differentiation of the homomorphic sex chromosomes in *Megaselia scalaris* (Diptera) detected by random DNA probes, *Chromosoma*, 99, 237, 1990.
210. **Traut, W. and Willhoeft, U.,** A jumping sex determining factor in the fly *Megaselia scalaris*, *Chromosoma*, 99, 407, 1990.
211. **Dübendorfer, A., Hilfiker-Kleiner, D., and Nöthiger, R.,** Sex determination mechanisms in dipteran insects: the case of *Musca domestica*, *Semin. Dev. Biol.*, 3, 349, 1992.
212. **Franco, M. G., Rubini, P. G., and Vecchi, M.,** Sex-determinants and their distribution in various populations of *Musca domestica* L. of western Europe, *Genet. Res.*, 40, 279, 1982.
213. **Tomita, T. and Wada, Y.,** Multifactorial sex determination in natural populations of the housefly (*Musca domestica*) in Japan, *Jpn. J. Genet.*, 64, 373, 1989.
214. **Jayakar, S. D.,** Some two-locus models for the evolution of sex-determining mechanisms, *Theor. Popul. Biol.*, 32, 188, 1987.
215. **Shono, T. and Scott, J. G.,** Autosomal sex-associated pyrethroid resistance in a strain of housefly (Diptera: Muscidae) with a male-determining factor on chromosome three, *J. Econ. Entomol.*, 83, 686, 1990.
216. **Denholm, I., Franco, M. G., Rubini, P. G., and Vecchi, M.,** Identification of a male determinant on the X chromosome of a housefly (*Musca domestica* L.) population in south-east England, *Genet. Res.*, 42, 311, 1983.
217. **Denholm, I., Franco, M. G., Rubini, P. G., and Vecchi, M.,** Geographical variation in house fly (*Musca domestica* L.) sex determinants within the British Isles, *Genet. Res.*, 47, 19, 1986.
218. **Kerr, R. W.,** Inheritance of DDT resistance in a laboratory colony of the house fly, *Musca domestica*, *Aust. J. Biol. Sci.*, 23, 377, 1970.
219. **Loeschke, V., Nielsen, B. O., and Andersen, D.,** Chromosomal variation, segregation and sex determination in *Hydrotaea meridionalis* (Diptera: Muscidae), *Hereditas*, 118, 229, 1993.
220. **Samoylov, Yu. B.,** [Genetic control for the cabbage root fly II. Localization of male determining factor in the cabbage root fly *Delia brassicae* Bouche], *Genetika*, 21, 1810, 1985.
221. **Inoue, H. and Hiroyishi, I.,** A maternal effect sex transformation mutant of the housefly *Musca domestica*, *Genetics*, 12, 469, 1986.
222. **Maddern, R. H. and Bedo, D. G.,** Properties of the sex chromosomes of *Lucilia cuprina* deduced from radiation studies, *Genetica*, 63, 203, 1984.
223. **Bedo, D. G. and Foster, G. G.,** Cytogenetic mapping of the male-determining region of *Lucilia cuprina* (Diptera: Calliphoridae), *Chromosome*, 92, 344, 1985.
224. **Ribbert, D.,** Die Polytänchromosomen der Borstenbildungszellen von *Calliphora erythrocephala*, *Chromosoma*, 21, 296, 1967.
225. **Ullerich, F.-H.,** Analysis of sex determination in the monogenic blowfly *Chrysomya rufifacies* by pole cell transplantation, *Mol. Gen. Genet.*, 193, 479, 1984.
226. **Clausen, S. and Ullerich, F.-H.,** Sequence homology between a polytene band in the genetic sex chromosomes of *Chrysomya rufifacies* and the *daughterless* gene of *Drosophila melanogaster*, *Naturwissenschaften*, 77, 137, 1990.
227. **Lifschitz, E. and Cladera, J. L.,** *Ceratitis capitata:* cytogenetics and sex determination, in *Fruit Flies, Their Biology, Natural Enemies and Control (World Crop Pests 3B)*, Robinson, A. S. and Harper, G., Eds., Elsevier, Amsterdam, 1989, 3.
228. **Solferini, V. N. and Morgante, J. S.,** $X_1X_1X_2X_2$:X_1X_2Y mechanism of sex determination in *Anastrepha bistrigata* and *A. serpentina* (Diptera: Tephritidae), *Rev. Bras. Genet.*, 13, 201, 1990.
229. **Grewal, J. S. and Kapoor, V. C.,** Karyotypes of some fruitfly species (Tephritidae) of India, in *Fruit Flies of Economic Importance (CEC/IOBC Int. Symp., Rome, 7–10 April 1987)*, Cavallaro, R., Ed., A. A. Balkema, Rotterdam, 1989, 237.
230. **Bush, G. L.,** Female heterogamety in the family Tephritidae (Acalyptrata, Diptera), *Am. Nat.*, 100, 119, 1966.
231. **Anleitner, J. E. and Haymer, D. S.,** Y-Enriched and Y specific DNA sequences from the genome of the Mediterranean fruit fly, *Ceratitis capitata*, *Chromosoma*, 101, 271, 1992.
232. **Hodgkin, J.,** *Drosophila* sex determination: a cascade of regulated splicing, *Cell*, 56, 905, 1989.
233. **Slee, R. and Bownes, M.,** Sex determination in *Drosophila melanogaster*, *Q. Rev. Biol.*, 65, 175, 1990.
234. **Torres, M. and Sanchez, L.,** The segmentation gene *runt* is needed to activate *Sex-lethal*, a gene that controls sex determination and dosage compensation in *Drosophila*, *Genet. Res.*, 59, 189, 1992.
235. **Steinmann-Zwicky, M.,** How do germ cells choose their sex? *Drosophila* as a paradigm, *BioEssays*, 14, 513, 1992.

236. **Cline, T. W.,** The *Drosophila* sex determination signal: how do flies count to two?, *Trends Genet.*, 9, 385, 1993.
237. **Steinemann, M. and Steinemann, S.,** Evolutionary changes in the organization of the major LCP gene cluster during sex chromosomal differentiation in the sibling species *Drosophila persimilis, D. pseudobscura* and *D. miranda, Chromosoma,* 99, 424, 1990.
238. **Ganguly, R., Swanson, K. D., Ray, K., and Krishnan, R.,** A *BamHI* repeat element is predominantly associated with the degenerating NEO-Y chromosome of *Drosophila miranda* but absent in *Drosophila melanogaster* genome, *Proc. Natl. Acad. Sci. U.S.A.*, 89, 1340, 1992.
239. **Steinemann, M., Steinemann, S., and Lottspeich, F.,** How Y chromosomes become genetically inert, *Proc. Natl. Acad. Sci. U.S.A.*, 90, 5737, 1993.
240. **Ohno, S.,** Evolution of sex chromosomes in mammals, *Annu. Rev. Genet.*, 3, 495, 1969.
241. **Jablonka, E. and Lamb, M. J.,** The evolution of heteromorphic sex chromosomes, *Biol. Rev. Camb. Philos. Soc.*, 65, 249, 1990.
242. **Charlesworth, D. and Charlesworth, B.,** Sex differences in fitness and selection for certain fusions between sex chromosomes and autosomes, *Genet. Res.*, 35, 205, 1980.