REVIEW

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# The evolution of sex chromosomes in insects: Differentiation of sex chromosomes in flies and moths*

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**Abstract.** Although a monophyletic group, male (XX/XY) and female heterogametic (WZ/ZZ) sex chromosome systems with a couple of variants like XX/X, Z/ZZ and multiple sex chromosome systems occur in insects. Molecular and morphological differences between X and Y or W and Z range from imperceptible to conspicuous. This article illustrates sex chromosome differentiation mainly in two fly species, *Drosophila melanogaster* and *Megaselia scalaris*, and in Lepidoptera. The earliest phases of XY evolution are present in the fly *M. scalaris*. Occasionally in this species, the male determining gene jumps to another chromosome, transforming the new host chromosome to a functional Y chromosome. Thus, in *M. scalaris* there are strains with virtually no XY differentiation (except for the sex determining function) and others with a moderate degree of differentiation. Base substitutions and alterations like sequence deletions, duplications, and insertions of mobile sequences mark the onset of molecular differentiation. Accumulation of molecular changes and coarser alterations are thought to lead to the morphological differences seen in WZ chromosome pairs of Lepidoptera. The W chromosome probably evolved in the most numerous clade of Lepidoptera, the Ditrysia, after it diverged from the common lepidopteran stem. Extant species display various degrees of molecular and morphological differentiation of the W chromosome, translocation or fusion with autosomes, and loss of the W.

## INTRODUCTION

Early in this century, Carl Correns (1907) investigated sex determination in a dioecious plant, *Bryonia dioica*. Using species hybrids, he found that inheritance of the two sex phenotypes followed the Mendelian backcross scheme (Fig. 1a). This has since proved to be the correct formal description of genetic sex determination in many but not all species. The scheme has several implications:

(1) There is an underlying genetic mechanism of sex determination. The formula, however, may not correctly represent either of the two cases in which the molecular basis of primary sex determination is known. In *Drosophila*, there are a couple of X-linked genes, which when present in double dose (on two X chromosomes) initiate female development in diploid embryos, when present in a single dose (on one X chromosome) male development (review: Cline & Meyer, 1996). Femaleness and maleness are not caused by different alleles of the same gene(s). In mammals, a single dose of *SRY* (alternatively designated *TDF*), is sufficient to initiate development of an embryo in the male direction. According to a hypothesis of Graves (1998) *SRY* was derived from *SOX3* and interacts with this X-chromosomal gene in primary sex determination. *SRY* and *SOX3* are not alleles in the strict sense, but they may at least share some properties of alleles.

(2) The backcross type of inheritance results in a 1 : 1 sex ratio in the progeny, generation after generation, and, hence, in the population, if no other influences prevail. A 1 : 1 sex ratio may have a biological advantage in some species. But Shaw & Mohler (1953) showed that selection favours it for different reasons. If females and males are not present at a 1 : 1 ratio, the mean genetic contribution to the next generation, of an individual from the underrepresented sex, is higher than that of one from the overrepresented sex. Hence, genetic factors that bring about the underrepresented sex, are bound to become more frequent in the next generation. This may explain the tendency in gonochoristic organisms to have mechanisms such as this backcross scheme that result in a stable 1 : 1 ratio.

(3) In reality, this scheme does not represent the transmission of a single Mendelian gene but of large segments of chromosomes or even whole chromosomes: the sex chromosomes. They are designated X and Y in systems with male heterogamety (Fig. 1b). In systems with female heterogamety, the sex chromosomes are given a different genetic notation, W and Z, but otherwise behave similarly (Fig. 1c). Insects, though monophyletic (Kristensen,

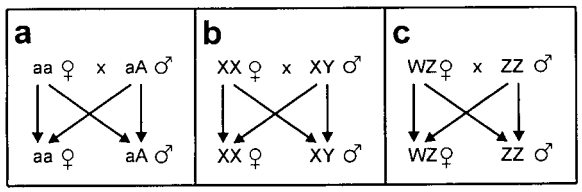


Fig. 1. a – the Mendelian backcross scheme; b – transmission of sex chromosomes in systems with male heterogamety; c – in those with female heterogamety.

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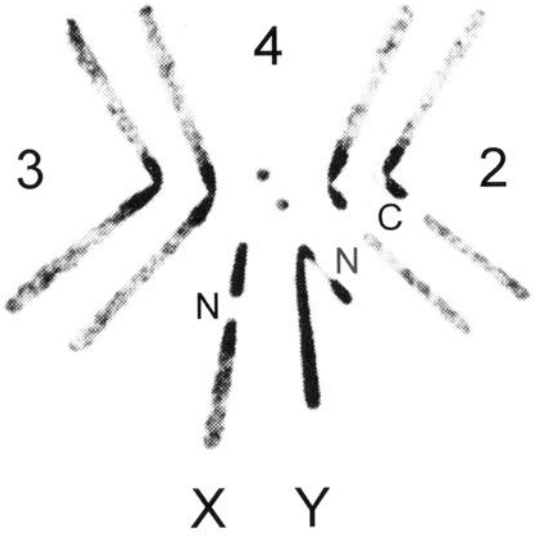


Fig. 2. Male karyotype of *Drosophila melanogaster*. Black – heterochromatin; N – nucleolus organizer region; C – prominent secondary constriction (from Heitz, 1934, explanations added).

1991) have both types of sex chromosome systems: Diptera, e.g., have heterogametic males, Lepidoptera have heterogametic females.

X and Y chromosomes may be morphologically alike ("homomorphic") or they are sufficiently different from one another ("heteromorphic") to be distinguished under a microscope. *Drosophila melanogaster*, e.g., has heteromorphic sex chromosomes: an acrocentric X and a smaller subacrocentric Y chromosome (Fig. 2). Old X and Y chromosomes such as those of *D. melanogaster*, the house mouse or man, differ not only in size and centromere position but also in their genetic composition. This can be seen from crossbreeding of sex chromosome-linked genes, sequencing of chromosomal DNA or, in a more general way, by comparative genomic hybridization (CGH; Kallioniemi et al., 1992), a variant of the fluorescence in situ hybridization technique that uses two differently labeled whole genomic DNAs as probes. For this specific purpose, labeled total DNA of males competes with differently labeled total DNA of females for hybridization to the chromosomes (Fig. 3a, b) (Traut et al., 1999). Besides showing the degree of sex chromosome differentiation, CGH can be used as a universal method for the identification of differentiated sex chromosomes (Traut et al., 1998).

Even the most distinct sex chromosomes must have, originally, come from a homologous pair of chromosomes. If so, what caused the pair of sex chromosomes to become different from one another and the sex chromosomes to differ from the autosomes? (1) Y (or W) chromosomes form (or part of them forms) a genomic compartment that does not recombine with the respective X (or Z) chromosome. The suppression of recombination is thought to be selectively favoured by the accumulation of sexually antagonistic genes on the sex chromosomes (Rice, 1987a). In some species, e.g. *D. melanogaster* and Lepidoptera, meiotic recombination is completely suppressed in the heterogametic sex. New Y (or W) chromosomes in such species, therefore, are non-recombining from the start. (2) Y (or W) chromosomes are constantly "heterozygous" owing to their role in sex determination and – consequently – their peculiar mode of transmission, with the Y in the male line and the W in the female line.

Thus, acquisition of the sex determining function and suppression of recombination are prerequisites for the chromosome pair to undergo differentiation. The evolutionary consequences for a non-recombining genomic compartment are (1) independence of molecular changes in the compartment from those in its homologue, (2) loss of its ability to repair by recombination, and (3) selection and neutral drift acting at the level of the entire compartment. The longterm consequences are molecular and morphological differentiation between X and Y, or W and Z. A conspicuous and well-studied aspect of Y chromosome differentiation is the loss of most genetic functions. The Y chromosome becomes genetically "inert". Mechanisms that might promote this loss are "Muller's ratchet" (Charlesworth, 1978; Muller, 1964), "genetic hitchhiking" (Rice, 1987b) and "background selection" (Charlesworth, 1994; Charlesworth et al., 1997).

Experimental proof of such changes is difficult to obtain. Circumstantial evidence, however, comes from studies of autosomes that have become part of the sex chromosome system recently, e.g. in *Drosophila miranda* by fusion of an autosome with the Y chromosome and in *D. americana americana* by fusion of an autosome with the X chromosome. In *D. miranda*, though still recognizable as homologous, the recently fused part has undergone molecular changes such as insertions of transposons and inactivation of larval cuticle protein genes (Steinemann & Steinemann, 1992; Steinemann et al., 1993) while in *D. a. americana*, gene inactivation has not taken place; i.e. the recent fusion has not so far had evolutionary consequences (Charlesworth et al., 1997). In the following, I present two cases in which the univalent sex chromosomes, Y or W, differ in age and, consequently, have reached different stages of differentiation. In the fly, *Megaselia scalaris*, new Y chromosomes arise in laboratory populations and thus the differentiation of new and moderately old XY pairs can be studied. In Lepidoptera, the W chromosome arose in the Ditrysia, a clade that comprises more than 98% of the extant species, and all observed differences in the WZ pair must have evolved since then.

## Y CHROMOSOMES IN *MEGASELIA SCALARIS*

The phorid fly *M. scalaris* has only three pairs of chromosomes. There is little or no meiotic recombination in the male, depending on the strain and the chromosome. Sex is determined by the absence or presence of a single *Maleness* factor. In the early sixties, C. Tokunaga in Japan and a group lead by F. Mainx in Vienna detected the *Maleness* factor in different linkage groups and its change to other positions by supposed translocation events (Mainx, 1964; Tokunaga, 1958). In a reinvestigation, we

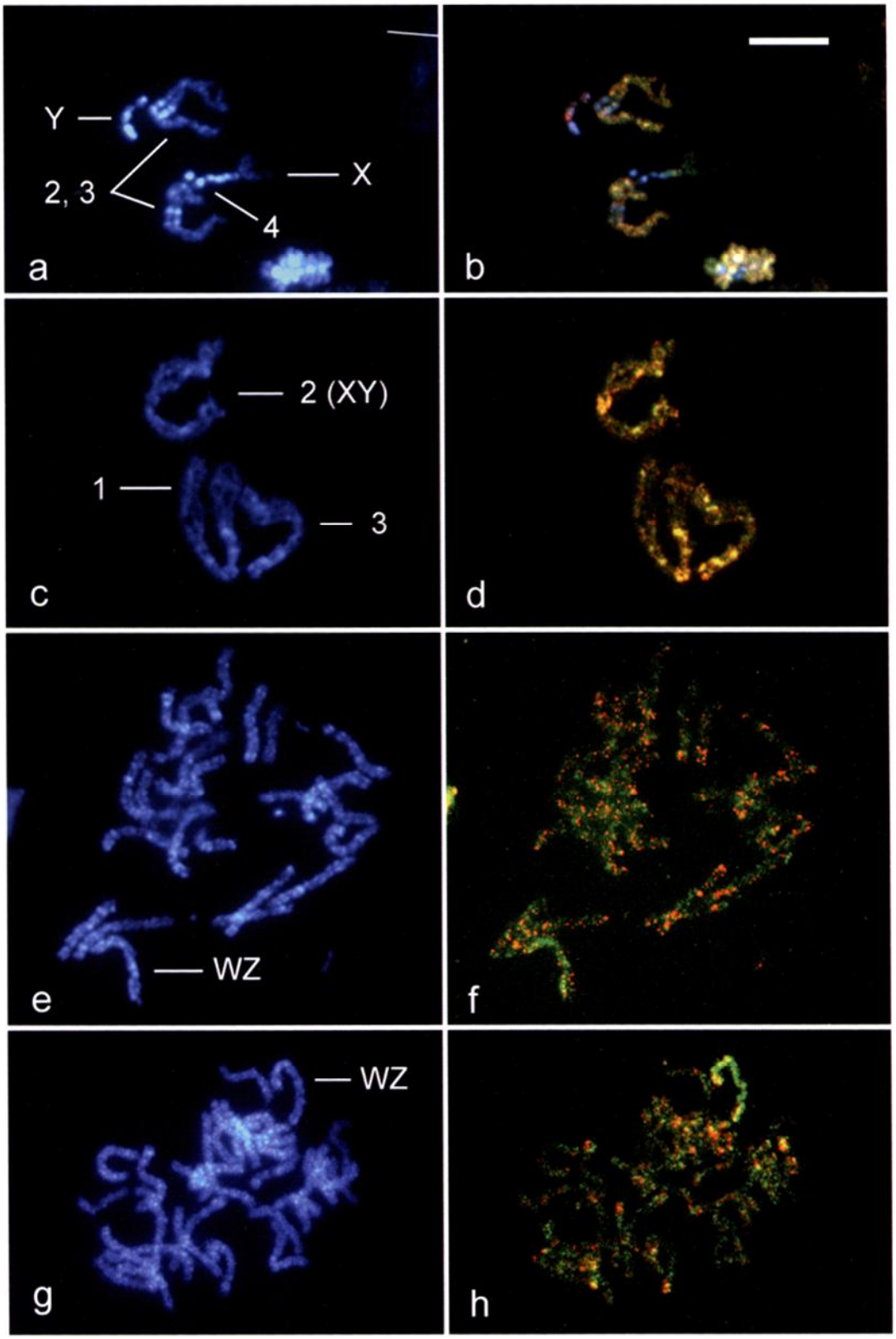


Fig. 3. Insect chromosomes after CGH (right panels) using FluoroX-labeled whole genomic DNA from females (green fluorescence) and Cy3-labeled DNA from males (red fluorescence), counterstaining (left panels) with DAPI (blue fluorescence). a, b – mitotic chromosomes of *D. melanogaster*. The centromeric heterochromatin is preferentially stained by DAPI (blue), the large autosomes II and III are painted equally by the female and male probe (yellow-brown), the Y chromosome only by the male probe (red), and the X preferentially by the female probe (greenish), due to the double concentration of X-chromosomal DNA in the female probe. c, d – mitotic chromosomes of male *Megaselia scalaris*. No chromosome is preferentially differentiated by the male or female probe. e, f – pachytene complement of *Ephestia kuehniella*. The W is highlighted by the female probe. g, h – pachytene complement of *Galleria mellonella*, the W chromosome highlighted by the female probe. Scale bar: 10 μm.

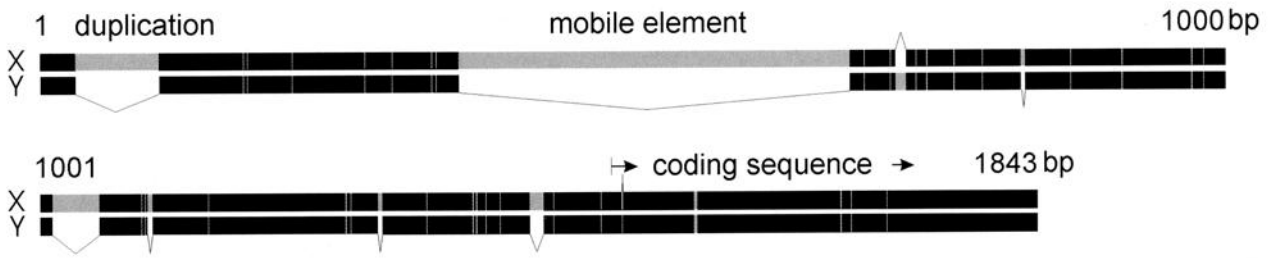


Fig. 4. A homologous segment of 1843 bp from the X and Y chromosomes of *Megaselia scalaris*, strain Wien. Black blocks: sequence identity between X and Y; shaded blocks: single nucleotide differences and differential segments; lines span missing homologous segments. The 3' end contains the beginning of the coding sequence for a vespid antigen 5-like protein. Data from Traut & Wollert (1998).

found transposition-like jumps of the *Maleness* factor at a rate of less than $10^{-3}$ (Traut & Willhoeft, 1990). Each jump creates a new Y chromosome. Exceptional males that resulted from such jumps, were used to establish new Y chromosome strains. The chromosomal assignment of the *Maleness* factor in these strains and, hence, the identity of the Y chromosome was determined with the aid of phenotypic and molecular markers (Traut, 1994).

Thus, a collection of strains with Y chromosomes of different origin is available: One derived from chromosome #1, two from chromosome #3 and five from chromosome #2, among them three strains, "Florida", "Tennessee" and "Wien", with Y chromosomes from natural populations (Table 1). Two new Y chromosomes were derived from previous X chromosomes. The Y chromosomes in these strains were not only derived from different chromosomes, they are also of different age. Some are quite young, as they arose during the experiments. Those isolated from natural populations are obviously older. There is additional circumstantial evidence that the Wien and Tennessee Y chromosomes are older. Molecular markers prove that the Tennessee and Wien Y chromosomes – but not the Florida Y chromosome – stem from the same transposition event. This must have occurred sufficiently long ago for them to spread to different localities (Willhoeft & Traut, 1990).

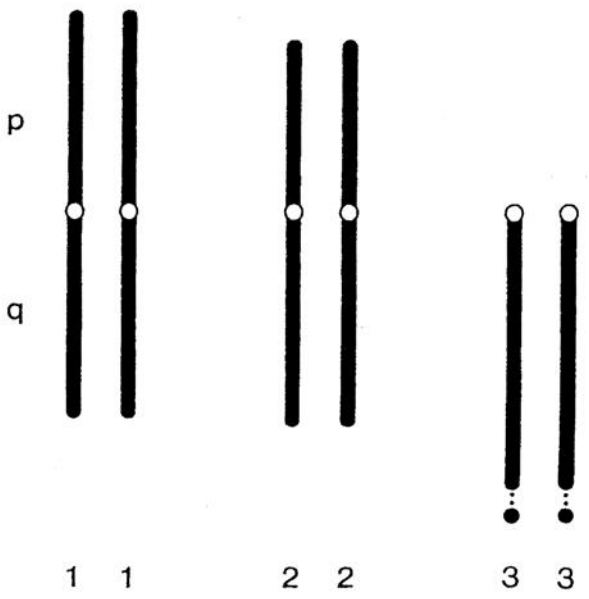


Fig. 5. An idiogram of a male *Megaselia scalaris*.

New and moderately old XY pairs of *M. scalaris* show different levels of molecular differentiation, as expected. There was only one molecular marker that distinguished X and Y in any of the new strains (Traut, 1994). It was a marker that detected a polymorphism before and a fixed difference after establishment of that particular Y chromosome strain. By contrast, in the Wien strain, more than 20 such markers are known (Willhoeft & Traut, 1995; Traut, unpubl.).

The X and Y chromosomal sequences from one of the markers were cloned and sequenced to disclose the differences between the two (Traut & Wollert, 1998). Both, the X and Y segment, contain the start of the coding sequence for a vespid Antigen5-like protein at one end. Not only in this region but also in the intergenic region, the sequences are sufficiently conserved for a reliable alignment (Fig. 4). This allows a clear definition of the differences. There are numerous single basepair substitutions and a few larger mutations. The larger mutations are sequence insertions and/or deletions. In this early stage of sex chromosome evolution, when sequences are still mostly identical, these larger mutations contribute more to the X/Y differentiation than the numerous single basepair exchanges. They disrupt homology in a rather coarse manner.

TABLE 1. Origin of the Y chromosome in laboratory strains of *Megaselia scalaris*. Each of the three chromosome pairs in *M. scalaris* can be the XY pair, depending on the location of the *Maleness* factor in the respective strain. Florida, Tennessee and Wien are wild-type strains from different geographical localities. The Except strains were derived from the Wien strain by breeding from exceptional males with deviant sex determination. The origin of the Y chromosome was determined by crossbreeding using phenotypic and molecular markers (Traut, 1994; Traut & Willhoeft, 1990).

| Strain | Y from chromosome |
|---|---|
| Except45 | #1 |
| Florida | #2 |
| Tennessee | #2 |
| Wien | #2 |
| Except11 | #2 (former X) |
| Except46 | #2 (former X) |
| Except1 | #3 |
| Except10 | #3 |

An inspection of the chromosomes does not detect morphological differences between X and Y, neither in the new nor in the moderately old Y chromosome strains. The sex chromosomes are homomorphic (Fig. 5). We did not expect to detect molecular differences by CGH between X and Y chromosomes in the new Y chromosome strains. But even in the moderately old Y chromosome strain, Wien, the molecular differences that were revealed by sequencing were not detectable by CGH (Fig. 3c, d) (Traut et al., 1999). The sex chromosomes have not acquired sufficient X- or Y-specificity in the period of time spent since their establishment. So, the stage of X/Y differentiation in the Wien strain is appropriately termed "early".

## THE W CHROMOSOME IN LEPIDOPTERA

Butterflies and moths, in contrast to flies, have heterogametic females: WZ/ZZ sex chromosome systems predominate (Traut & Marec, 1997). Mitotic chromosomes in Lepidoptera are usually small and numerous, with chromosome numbers around 2n = 60 (Robinson, 1971). Meiotic chromosomes, especially at the pachytene stage when homologues are long and fully synapsed, are best suited to study morphological details, e.g. with respect to W/Z differentiation.

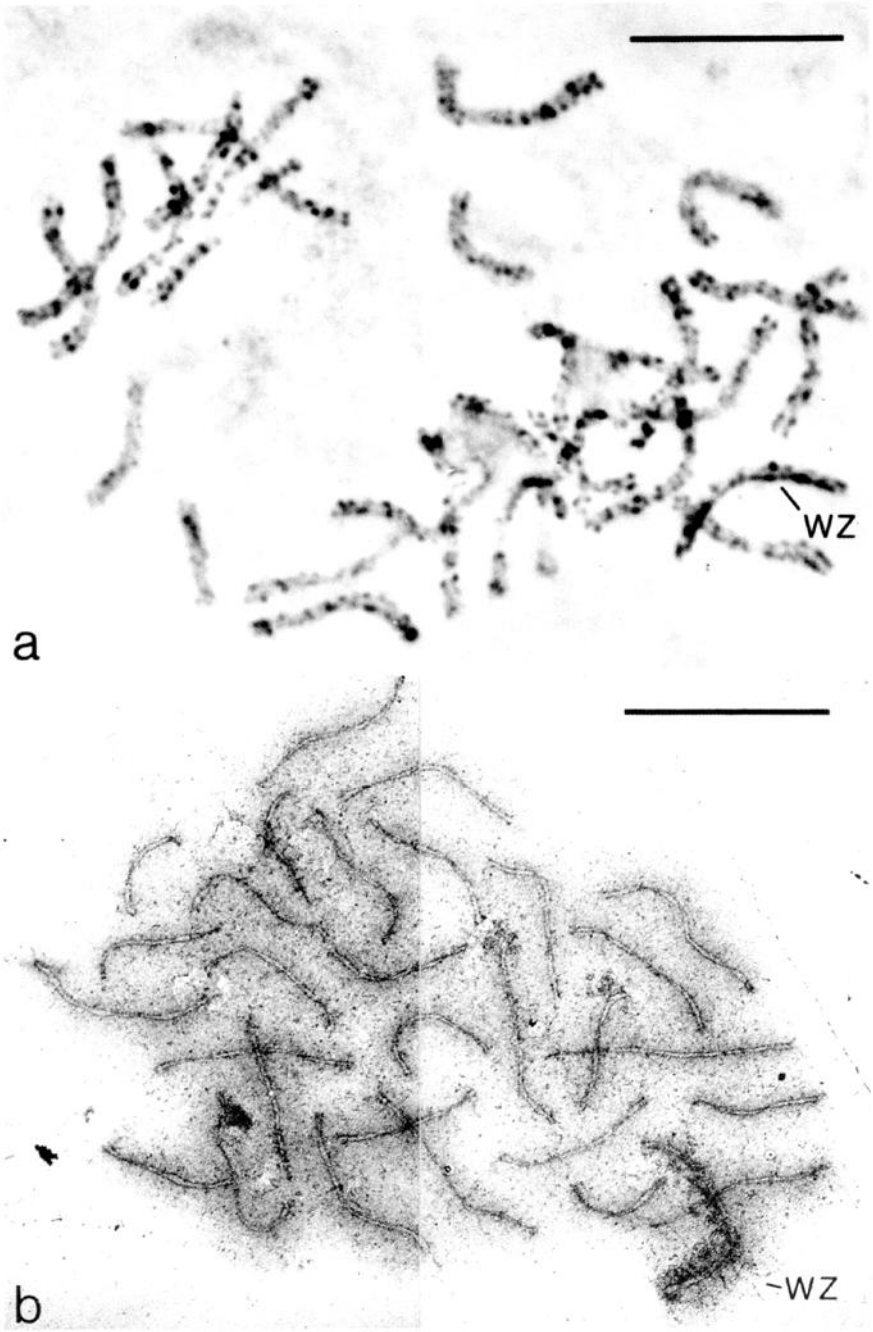


Fig. 6. *Ephestia kuehniella*, pachytene complement of a female (from Traut et al., 1986). a – light microsopic preparation; b – synaptonemal complexes with associated chromatin, EM. Scale bar: 10 μm.

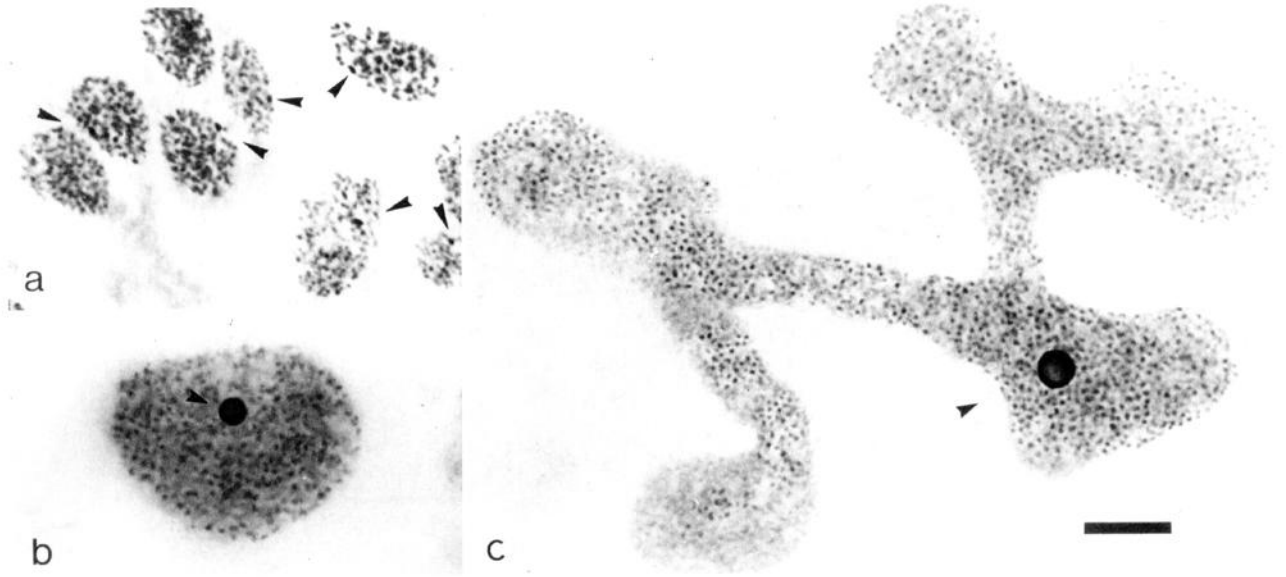


Fig. 7. Sex chromatin (arrowheads) in female somatic cells of an *Ephestia kuehniella* larva. a – diploid cells from a wing imaginal disc; b – polyploid nucleus from the neck of a Malpighian tubule; c – highly polyploid nucleus from a Malpighian tubule. Scale bar: 10 μm.

In meiosis in female flour moths, *Ephestia kuehniella*, the WZ bivalent can be distinguished from autosomal bivalents (Fig. 6). The paired autosomes display a fairly homologous chromomere pattern. W and Z chromosomes, in contrast, have completely different patterns. The Z chromosome shows chromomeres alternating with interchromomeres while the W chromosome is a conspicuous intensively staining heterochromatic thread (Traut & Rathjens, 1973). In spite of these differences, W and Z synapse throughout their length. Synapsis does not result in recombination though; there is no meiotic recombination in female Lepidoptera (Traut, 1977).

Sex chromosome morphology at the pachytene stage has been investigated in several moth and butterfly species (Traut & Marec, 1997). In the majority, differences in chromomere pattern mark the WZ bivalent; in others, no such difference is visible. Thus, many lepidopteran WZ pairs are morphologically differentiated at this level of resolution. In some species, size differences are apparent in electron microscopy studies of the pachytene (Wang et al., 1993; Weith & Traut, 1980) and occasionally also in conventional mitotic spreads when the chromosomes of a species are exceptionally small or large (Traut & Mosbacher, 1968). Morphological differentiation of sex chromosomes is most obvious in some species with multiple sex chromosome systems like $W_1W_2Z/ZZ$ or $WZ_1Z_2/Z_1Z_1Z_2Z_2$, which result from sex chromosome fission and/or fusion events with autosomes (Nilsson et al., 1988; Suomalainen, 1969). In other species, the W chromosome is missing, the sex chromosome system is Z/ZZ (Ennis, 1976; Traut & Mosbacher, 1968). In the context of sex chromosome differentiation, these are extreme cases.

Molecular differentiation of the WZ pair of chromosomes in *E. kuehniella* is apparent when the pachytene chromosomes are submitted to CGH with the DNA from females and males differently labeled (Traut et al., 1999). The female DNA probe highlights the W chromosome (Fig. 3e, f). In the wax moth, *Galleria mellonella*, where the WZ bivalent can be identified using electron microscopy (Wang et al., 1993) but not light microscopy (Traut, unpubl.), CGH reveals molecular differentiation between W and Z over most of their length (Fig. 3g, h). These lepidopteran W chromosomes are obviously in an advanced stage of molecular differentiation.

Partial or total heterochromatinization is another feature of lepidopteran W chromosomes (Traut & Marec, 1997), and sex chromosomes generally (White, 1973). In the somatic cells of Lepidoptera, W chromosomes form conspicuous heterochromatic masses. Due to its derivation from W and its female-specific presence the heterochromatin is designated "W chromatin" or "sex chromatin". Size and/or number of sex chromatin bodies increase with increasing levels of polyploidy (Fig. 7). Thus, when presence or absence of W chromosomes is to be looked for in a larger number of species, it is best done by studying sex chromatin in polyploid somatic nuclei.

In a compilation of data from 238 species, more than 80% of all species contained sex chromatin (Traut & Marec, 1996). Possession of sex chromatin, therefore, is a characteristic trait of Lepidoptera. Arranged in a cladogram, the data reveal a difference between families of the basal lepidopteran clades (non-ditrysian families) and those belonging to the Ditrysia (Fig. 8). All ditrysian families include species with sex chromatin and sporadically species without sex chromatin. In contrast, none of the species investigated from three non-ditrysian families had sex chromatin. They share this character with the closest related group, the Trichoptera (Marec & Novák, 1998). The inference is that the W chromosome had not evolved when those non-ditrysian lineages diverged from the common lepidopteran stem. In one of the non-

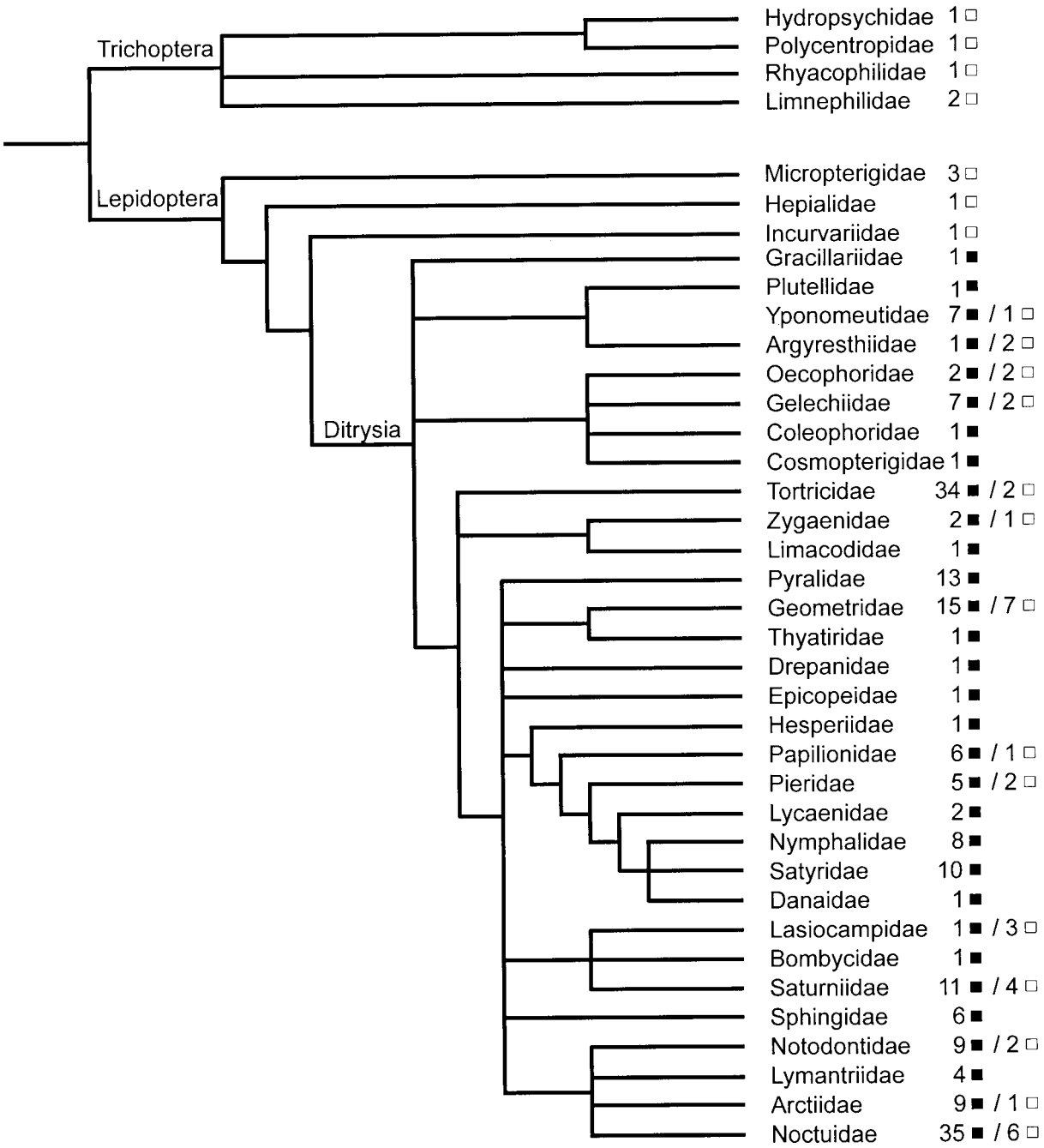


Fig. 8. Cladogram of trichopteran and lepidopteran families. Numbers of studied species with (■) and without (□) sex chromatin. The phylogenetic tree was adapted from Nielsen & Common (1991) and Morse (1997), and the data from Marec & Novák (1998) and Traut & Marec (1996).

ditrysian lepidopteran species, *Micropterix calthella*, absence of the W chromosome has been confirmed: the Z chromosome is a univalent in female meiosis (Traut & Marec, 1997).

In conclusion, Lepidoptera acquired their W chromosome after at least those three non-ditrysian lineages had separated. A possible scenario for the acquisition of the W is the fusion of an autosome with the Z chromosome. The free homologue of the fused autosome is then transmitted as a W chromosome in the female lineage. Whatever the source of the W chromosome, it forms a non-recombining genetic compartment in the genome since lepidopteran females lack meiotic recombination. All molecular and morphological differentiation of the WZ pair must have taken place since then, including the loss of the W chromosome in some ditrysian species. Thus, the lepidopteran W chromosome can be traced from its origin, through molecular and morphological differentiation, to its disappearance.

## SEX CHROMOSOME SYSTEMS IN INSECTS

Chromosomal sex determination allows only for a limited amount of variation in the sex chromosome system, in insects as well as in other organisms: XY/XX, XX/X,

TABLE 2. Sex chromosome systems in insects. Largely based on Blackman (1995) and White (1973).

| | ♀♀ | ♂♂ |
|---|---|---|
| Collembola | XX | X |
| | XX | XY |
| Ephemeroptera | XX | XY |
| | XX | X |
| Odonata | XX | X |
| | XX | XY |
| Plecoptera | XX | X |
| | $X_1X_1X_2X_2$ | $X_1X_2$ |
| | $X_1X_1X_2X_2X_3X_3$ | $X_1X_2X_3$ |
| Blattodea | XX | X |
| Isoptera | XX | XY |
| | XX | X |
| | multiple sex chromosomes | |
| Mantodea | XX | X |
| | $X_1X_1X_2X_2$ | $X_1X_2Y$ |
| Dermaptera | XX | XY |
| | XX | X |
| | multiple sex chromosomes | |
| Grylloblattodea | XX | XY |
| Orthoptera | XX | X |
| | XX | XY |
| | $X_1X_1X_2X_2$ | $X_1X_2Y$ |
| Phasmatodea | XX | X |
| | XX | XY |
| Embioptera | XX | X |
| Psocoptera | XX | X |
| Hemiptera | XX | XY |
| | XX | X |
| | multiple sex chromosomes | |
| Coleoptera | XX | XY |
| | XX | X |
| | multiple sex chromosomes | |
| Megaloptera | XX | XY |
| Raphidioptera | XX | XY |
| Neuroptera | XX | XY |
| Trichoptera | Z | ZZ |
| Lepidoptera | WZ | ZZ |
| | Z | ZZ |
| | $W_1W_2Z$ | ZZ |
| | $WZ_1Z_2$ | $Z_1Z_1Z_2Z_2$ |
| Mecoptera | XX | X |
| | $X_1X_1X_2X_2$ | $X_1X_2Y$ |
| Siphonaptera | XX | XY |
| | multiple sex chromosomes | |
| Diptera | XX | XY |
| | XX | X |
| | $X_1X_1X_2X_2$ | $X_1X_2$ |

WZ/ZZ, Z/ZZ and multiple sex chromosome systems (Table 2). Only Lepidoptera and Trichoptera have female heterogamety, in all others the male is heterogametic (with some exceptions). Multiple sex chromosomes have evolved independently in several insect orders, by fusion or reciprocal translocation between autosomes and sex chromosomes (White, 1973). The most complex case is that of a race of the termite, *Kalotermes approximatus*, where ten different X chromosomes and nine different Y chromosomes are present in the male genome (Syren & Luykx, 1981). Loss of the constantly univalent sex chromosome, Y or W, has occurred in both systems. In fact, most insects are in this stage of sex chromosome evolution. This throws some light on the predominant sex determining mechanism among insects: as in *Drosophila*, double vs. single doses of the X (or Z) determine sex in these species. XX/X or Z/ZZ systems are ultimate stages of sex chromosome differentiation but are not dead ends of sex chromosome evolution. Fusion or translocation of an autosome creates a new Y or W as in Lepidoptera. This has happened frequently in diverse insect groups and was studied extensively in Orthoptera (review: White, 1973). It starts another cycle of differentiation and degeneration of a univalent sex chromosome.

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