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Genetics of sex determination: what can we learn from *Drosophila*?

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Summary

The combined efforts of genetics, developmental and molecular biology have revealed the principles of genetic control of sexual differentiation in *Drosophila*. In combination with maternal components, a quantitative chromosomal signal, provided by the ratio of X chromosomes to sets of autosomes (X:A), regulates a key gene (*Sxl*). The functional state, ON or OFF, of *Sxl*, *via* a few subordinate regulatory genes, controls a switch gene (*dsx*) that can express two mutually exclusive functions, M or F. These serve to repress either the female or the male set of differentiation genes, thus directing the cells either into the male or into the female sexual pathway.

Investigations of control genes and their regulation show that they have properties of homeotic genes. Their role is to select one of two alternative developmental programs. Their function, or lack of function, is required throughout development to maintain the cells in their respective sexual pathway.

Differentiation genes are under negative control by *dsx*. We discuss the *cis*- and *trans*-regulatory elements that are needed for sex-, tissue- and stage-specific expression of the differentiation genes.

A comparison of *Drosophila* to other organisms such as *Caenorhabditis*, mammals and other insects indicates similarities that we interpret as evidence for a basically invariant genetic strategy used by various organisms to regulate sexual development.

Key words: *Drosophila*, sex determination, genetic control.

Introduction

Genetic, developmental and molecular analyses have revealed the principles governing sexual differentiation in *Drosophila*. A chromosomal signal, the ratio of X chromosomes to sets of autosomes (X:A), is used to regulate a small number of control genes whose state of activity instructs the differentiation genes to produce the morphological, physiological and behavioural differences that distinguish males from females.

Since sex determination in *Drosophila* has been extensively reviewed (Baker & Belote, 1983; Nöthiger & Steinmann-Zwicky, 1985*a*; Cline, 1985), we will only give a brief outline here. Our main focus will be on the regulation of the differentiation genes that respond to the sex-determining genes to produce the actual differences between the sexes. We will also discuss how *Drosophila* can serve as a paradigm for other organisms.

The regulatory pathway from the X:A signal to *dsx*

The regulation of Sxl

The key gene in the sex-determining pathway is *Sxl* (*Sex-lethal*; Cline, 1978) whose function also regulates the process of dosage compensation. *Sxl* is active in XX animals where it determines the female pathway and a low rate of transcription of the X chromosomes. In XY animals, it is inactive and this implements the male pathway and a high rate of X-chromosomal transcription (Cline, 1978; Lucchesi & Skripsky, 1981).

The state of *Sxl*, ON or OFF, is set around the blastoderm stage and thereafter becomes independent of the X:A signal (Sanchez & Nöthiger, 1983; Cline, 1984). Genetic analyses show that *Sxl* is regulated by maternal and zygotic elements. The maternal products, specified by the genes *da* (*daughterless*; Cline, 1976, 1978), and *Dk* (*Daughter-killer*; M. Steinmann-Zwicky, E. Fuhrer-Bernhardsgrütter, D. Franken & R. Nöthiger, unpublished data), are necessary

for *Sxl* to become active. Since they are produced by the mother, they are present in every zygote, male and female, and therefore cannot act as discriminators, but they provide a prerequisite for *Sxl* to become active. Mutations in these genes are lethal for daughters whose X chromosomes are now hyperactive; under certain conditions, a masculinizing effect upon XX progeny can be demonstrated (Cline, 1983; Steinmann-Zwicky *et al.* 1987).

The zygotic elements are genetically less well defined. Bridges (1921) has shown that a quantitative signal, formed by the number of X chromosomes relative to the number of sets of autosomes, acts as the discriminator (1X;2A = male, 2X;2A = female, 2X;3A = intersexual). In normal females and males, the X:A ratio decides whether *Sxl* is active or inactive. Two X-linked genetic elements seem to play major roles in the activation of *Sxl*: region 3E–4F (Steinmann-Zwicky & Nöthiger, 1985*a*) and *sis-a* (*sisterless-a*; Cline, 1988). These elements might act directly or indirectly to promote expression of *Sxl*. One model postulates that X-chromosomal sites bind and neutralize an autosomal repressor for *Sxl*, present in limited amounts (Chandra, 1985). In females with two X chromosomes, all repressor molecules are bound so that *Sxl* can be active; in males, however, the single X chromosome binds only half as many repressor molecules, leaving enough of them to repress *Sxl*.

The dsx-*locus and its control*

The sexual phenotype ultimately depends on the differential activity of *dsx* (*double sex*), a complex locus that can express two functions, dsx^m or dsx^f. The product of dsx^m, M, specifies the male pathway, that of dsx^f, F, the female pathway. Mutations that abolish the functions of *dsx* produce an intersexual phenotype.

Sxl does not directly control *dsx*, but instead uses at least three genes as intermediaries. These genes, *tra-2* (*transformer-2*), *tra* (*transformer*) and *ix* (*intersex*), are active when *Sxl* is active and then regulate *dsx* in such a way that it expresses the female-determining F function. When *Sxl* is inactive, *tra-2*, *tra* and *ix* are also silent; and in the absence of products from all these genes, *dsx* expresses the male-determining M function. This, then, represents the basic state of the *dsx*-locus. The locus acts as a double-switch that expresses one of two mutually exclusive functions used to implement either the male or the female sexual pathway. Mutations that destroy the function of *tra* or *tra-2* result in XX animals being transformed into sterile males (pseudomales). This shows that absence of tra^+ or $tra\text{-}2^+$ leaves dsx^+ in the basic, male-determining state. When tra^+ and $tra\text{-}2^+$ are active, but *ix* is mutant, XX animals develop as intersexes of the same type as when *dsx* is nonfunctional. We conclude that tra^+ *and* $tra\text{-}2^+$ cooperate to prevent *dsx* from expressing the M function, and that ix^+ adds whatever is needed for *dsx* to express the F function (Nöthiger *et al.* 1987).

From the mutant phenotypes, we can deduce the functional state, ON or OFF, of these genes in wild-type males and females; and the epistatic relations observed in double mutants allow us to order the genes in a functional sequence (Fig. 1).

Molecular analysis

The sex-determining genes are currently being cloned. The reports published show that the genes are

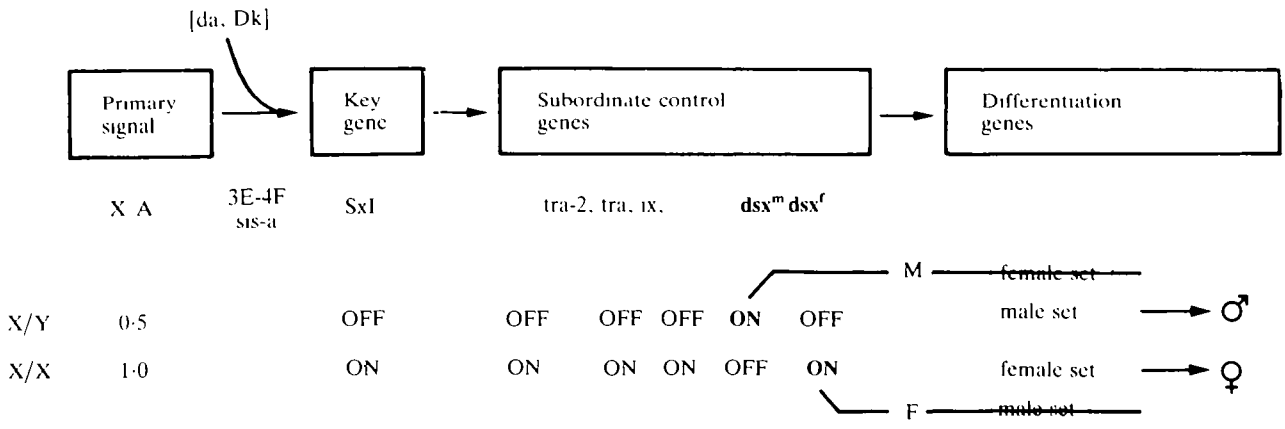

Fig. 1. The genetic hierarchy regulating sexual differentiation in *Drosophila*. The primary signal for sexual differentiation is the ratio of X chromosomes to sets of autosomes (X:A). This chromosomal signal is the discriminator that initiates the male or the female pathway. Zygotic (region 3E–4F, *sis-a*) and maternal (*da*, *Dk*) components regulate the key gene *Sxl* whose state of activity, symbolized by ON or OFF, achieves the implementation of one of two complementary activity patterns at the subordinate control genes. The mutually exclusive products of dsx^m and dsx^f, M or F, finally serve to repress either the female or the male differentiation genes, thus producing either a male or a female.

variable in size: 32 kb for *Sxl* (Maine, Salz, Cline & Schedl, 1985*a*,*b*), 30 kb for *dsx* (Belote *et al.* 1985*b*) and as little as 2 kb for *tra* (Butler, Pirrotta, Irminger-Finger & Nöthiger, 1986; McKeown, Belote & Baker, 1987).

So far, only the *tra* gene has been subjected to a functional test. It was inserted into a transposable vector and transgenic flies were produced (Butler *et al.* 1986; McKeown *et al.* 1987). A DNA fragment of 3·8 kb, integrated anywhere in the genome, completely rescued *X/X; tra/tra* zygotes, which now developed as fertile females. Other experiments showed that an even smaller fragment of only 2 kb contains the essential functions of *tra*. When this short stretch of DNA is defective, an avalanche of consequences ensues, so that chromosomal (XX) females develop as males.

The technique of genetic transformation makes it possible to manipulate the cloned genes *in vitro*, to reintroduce them into the genome and to study the effects of such constructs *in vivo*. Constitutive promoters may be used to express sex-determining genes in the wrong sex. Such experiments will reveal whether a hierarchy exists among the sex-determining genes and, if so, how they are ordered and regulated.

The gene *dsx* is required in both sexes, but the other sex-determining genes seem to be exclusively needed to produce a female. The transcriptional pattern of some of these genes is disturbingly complex. In their preliminary analysis of *Sxl*, Maine *et al.* (1985*b*) describe ten different transcripts, some of which are only found in females, others in both sexes, and still others are male-specific. This latter class was unexpected since X/Y flies lacking the entire *Sxl* gene are fertile males, which suggests that the gene is only required in females. The male-specific transcripts might have a negative regulatory function on *Sxl*, namely to prevent the production of female-determining transcripts (Maine *et al.* 1985*b*). This is a reasonable hypothesis since we have seen that the state of activity of *Sxl* is irreversibly fixed during embryonic development, after which period it becomes independent of the primary X:A signal (Sanchez & Nöthiger, 1983). Alternatively, male-specific transcripts might be required in males for some phenotypic trait that is not as obvious as sex, viability and fertility. Indeed, males deficient for *Sxl* are apparently unable to distinguish between males and females and therefore court flies of both sexes (Tompkins, 1986).

The number of sex-determining genes appears to be small. In a few years, we should be able to describe the process of sex determination in molecular terms. We shall see how the regulatory genes interact and whether this interaction occurs at the transcriptional or post-transcriptional level.

Sxl, tra-2, tra, ix *and* dsx *are homeotic genes whose cell-autonomous functions are required throughout development*

Genetic mosaics consisting of XX and XO cells are gynandromorphs in which XX cells develop female structures, and XO cells develop male structures side by side (Sturtevant, 1929). These animals reveal that sexual differentiation in *Drosophila* is cell autonomous, each cell differentiating according to its own sexual genotype.

Another type of mosaic is produced by mitotic recombination. The experimenter can induce this process by X-rays any time during development in a population of dividing cells. The result is a clone of homozygous mutant cells generated in a heterozygous animal. When the wild-type Sxl^+ allele was eliminated from a cell of a female larva that was heterozygous X/X; Sxl^-/Sxl^+, the resulting clone of Sxl^-/Sxl^- cells developed male structures (Sanchez & Nöthiger, 1982). The same result was obtained for *tra* and *tra-2* (Baker & Ridge, 1980; Wieschaus & Nöthiger, 1982) and it was also found that the product of dsx^+ is required until the very end of development (Baker & Ridge, 1980). These results show that the developmental history of a cell is irrelevant for its sexual phenotype. What matters is the actual genotype of the cells at the time of sexual differentiation. The state of activity of the sex-determining genes is continuously used to maintain the cells in their sexual pathway. Changing the genotype from an active Sxl^+ to an inactive Sxl^- even late in larval development has a domino effect on the subordinate control genes and on the differentiation genes, producing a switch from the female to the male pathway. This flexibility operates in both directions, from female to male and from male to female, as demonstrated by experiments with the temperature-sensitive allele $tra\text{-}2^{ts}$. XX animals homozygous for $tra\text{-}2^{ts}$ develop as males at 29°C whereas at 16°C they become females. Temperature shifts at different times of development showed that the sexual phenotype depended on the temperature applied during the period preceding the assay (Belote & Baker, 1982; Epper & Bryant, 1983). We will see that, within limits, 'sex reversal' is possible even in adult flies (Belote *et al.* 1985*a*). Thus, although with respect to karyotype sex determination occurs at fertilization, the sexual pathway remains flexible until final differentiation takes place.

The sex-determining genes have properties of homeotic genes. Like these, their alternative functional states, ON or OFF, specify alternative developmental programs; and like these, their state of activity is required throughout development to keep the cells

on the chosen pathway. The difference is that the sex-determining genes operate in *genetic* compartments, XX or XY, rather than in *spatial* domains as the homeotic genes do.

The differentiation genes

The differentiation genes are under control of the sex-determining genes

Mutations that abolish the function of *dsx* produce an intersexual phenotype at the cellular level. This is most clearly seen in the sex comb, a row of heavy blunt bristles on the basitarsus of the forelegs of males; in females, the bristles of this row are slender and pointed. In X/X; dsx^-/dsx^- and X/Y; dsx^-/dsx^- flies the shape of these bristles is intermediate between the male and female type (Fig. 2). The observations suggest that, in these intersexes, both male and female differentiation genes are expressed in the same cell. We, therefore, conclude that these genes are under negative control by *dsx*. The M function of *dsx* serves to repress the female set, the F function to repress the male set of sex-differentiation genes (Fig. 1) (Baker & Ridge, 1980; Nöthiger *et al.* 1987).

Genes required for the differentiation of female or male structures are largely unknown. The genes coding for the chorion proteins and yolk proteins, however, have been extensively analysed and can serve as a paradigm for female-specific differentiation genes. *Drosophila* produces three different yolk polypeptides, YP1, YP2 and YP3, that are encoded by a cluster of three genes on the X chromosome (Barnett, Pachl, Gergen & Wensink, 1980). These three genes are only active in the fat body (Gelti-Douka, Gingeras & Kambysellis, 1974) and the ovarian follicle cells (Brennan, Weiner, Goralski & Mahowald, 1982) of females; they are not noticeably transcribed in males. When pseudomales were assayed for YP, they were found to be negative like normal males. Intersexual flies, however, produced YP although in smaller amounts than normal females. The presence of YP in X/Y; dsx^-/dsx^- intersexes shows that YP production in these mutants is independent of the X:A ratio, but is controlled by the genes regulating the sexual pathway (Postlethwait, Bownes & Jowett, 1980; Bownes & Nöthiger, 1981). The same principle applies to morphological sexual characters (Steinmann-Zwicky & Nöthiger, 1985*b*).

A particularly illuminating example of this control is provided by the expression of the YP genes in X/X; $tra\text{-}2^{ts}/tra\text{-}2^{ts}$ animals. Depending on the temperature, such animals develop into male flies (at 29°C) or female flies (at 16°C). When adult pseudomales of this genotype (raised at 29°C) are shifted down to 16°C, the YP genes in the fat body start transcription within hours, which is followed by synthesis of yolk polypeptides. Conversely, when $tra\text{-}2^{ts}$ females (raised at 16°C) are brought to the restrictive temperature of 29°C, transcription of the YP genes gradually ceases (Belote *et al.* 1985*a*). We see that expression of the YP genes by the fat body requires a functional product of *tra-2*. The experiments also demonstrate the flexibility of the sexual pathway, since even adult tissue, here the fat body, can switch from one sex to the other.

High doses of ecdysterone, injected into normal adult males, can release the YP genes from repression

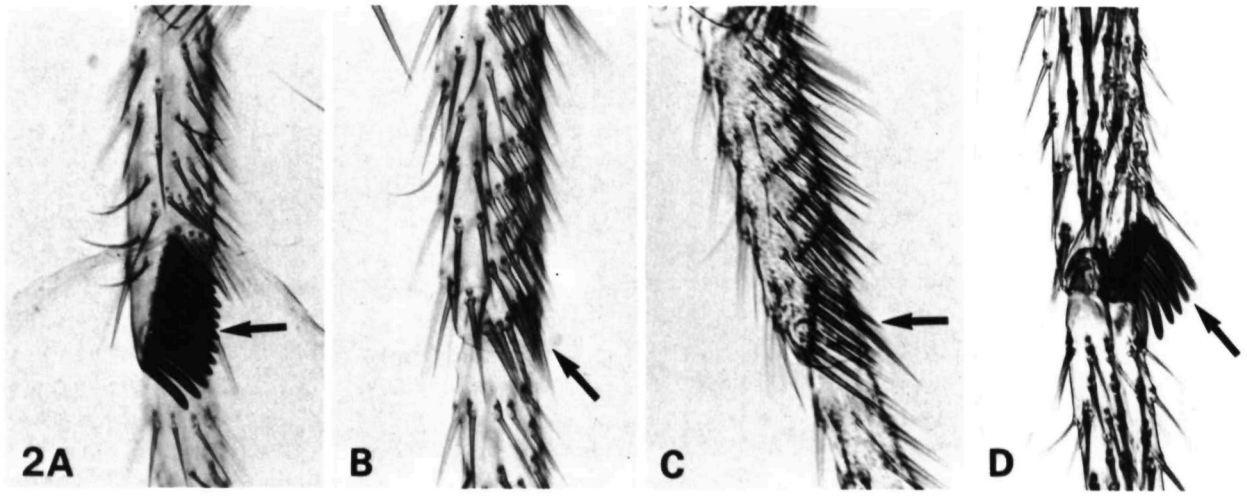

Fig. 2. Sex dimorphism on the foreleg. (A) On the male basitarsus the basal bristle row consists of heavy, blunt bristles, the sex comb, that is rotated relative to the other rows of bristles. (B) In females, the corresponding row runs parallel to the others and has slender, sharp bristles. (C) Flies mutant for *dsx* display an intersexual phenotype: the bristles are slightly heavier than in females and form a row that is partially rotated. (D) A clone of X/X; tra/tra cells generated in a heterozygous female shows cell-autonomous differentiation. Note that only six sex comb bristles are differentiated; the rest of the basal row consists of heterozygous tra/tra^+ cells which differentiate the female pattern.

by M, the product of dsx^m. The hormone does not achieve its effect by acting on the sex-determining genes since the same response is obtained from pseudomales, including X/X; dsx^D/dsx^- in which the M function is constitutively expressed (Bownes & Nöthiger, 1981). Whereas the sex-specific repression of the YP genes can be overridden by ecdysterone, the tissue-specific response remains intact and only the fat body produces some YP.

Tissue-specific expression

The genetic signal for 'maleness' (M produced by dsx^m) or 'femaleness' (F produced by dsx^f) is most probably the same in all cells of an animal. The phenotypic consequences, however, are very different for different cells: M in the brain leads to male behaviour, in the basitarsus of the foreleg to the production of a sex comb, in abdominal segment 9 to male genitalia, in the fat body it prevents synthesis of YP and for cells without sexual dimorphism, M or F apparently has no meaning. We recognize a classical concept of developmental biology: the specific response of a cell is not determined by the signal, but by the cell's developmental fate. In our current view, this fate, at least in *Drosophila*, is specified by the integrated action of maternal, segmentation and homeotic genes (for review see Mahowald & Hardy, 1985).

This principle is demonstrated by the phenotype of the homeotic mutation *Pc* which transforms the meso- and metathoracic legs into prothoracic legs. In males, which normally have a sex comb only on the forelegs, the transformation leads to the appearance of sex combs on all six legs. The example shows that the sexual phenotype, as we observe it, is the result of an interaction between an ubiquitous genetic signal, M or F, and a particular combination of active and inactive homeotic genes that assign the segmental and tissue-specific characteristics to the cells.

In its action, the sex-determining signal of *Drosophila*, M or F, is analogous to the sex-hormones of vertebrates. Whereas the sex hormones of vertebrates are synthesized by specific cell types in the gonad from where they flood the organism, in *Drosophila*, M and F are cell-autonomous products of a sex-determining gene. The result, in both cases, is that the signals are ubiquitous, reaching every cell; and in both cases the specificity of the response depends on the cell type.

Cis- *and* trans-*regulatory elements*

Several laboratories have begun to study the regulation of sex-specifically expressed genes whose protein products and functions are sometimes known, e.g. the yolk proteins (YP) or the chorion proteins; sometimes, the genes are only defined by sex-specific transcripts (Schäfer, 1986; DiBenedetto *et al.* 1987). We now want to discuss some experiments dealing with the problem of tissue-, stage-, and sex-specific expression of genes.

The DNA-sequences responsible for the expression of yolk proteins (YP) are present in all cells. But only two cell types, the fat body and follicle cells of adult females, can activate their YP genes. The tissue-specific control requires a *trans*-acting element which might function as an activator for the YP genes. Such a molecule would have to be synthesized in the particular cell type (fat body or follicle cells) at the proper time. In this way, tissue- and stage-specificity would be linked, making it unnecessary to postulate different control elements for the spatial and temporal aspects of gene expression.

The tissue-specific, *trans*-acting molecule has to recognize the gene to be regulated. For the gene encoding YP1, Wensink and coworkers (Shepherd, Garabedian, Hung & Wensink, 1985; Garabedian, Shepherd & Wensink, 1986) identified two discrete *cis*-acting sequences at the 5′ end of the gene. One of the sequences is necessary for expression in the fat body, the other for expression in the ovarian follicle cells. When these sequences were deleted, the gene could not be expressed. These *cis*-elements may serve as binding sites for a positive regulator of transcription provided by the fat body cells and the follicle cells.

To explain why the YP genes are not expressed in the fat body of males, a simple hypothesis, consistent with the previously deduced functions of *dsx*, is that the M product of dsx^m is a negative regulator that renders the *cis*-regulatory sites of female-specific differentiation genes inaccessible for the tissue-specific *trans*-activating factor.

Nothing is known about *trans*-acting tissue-specific control molecules, except that their interaction with the *cis*-sequences of the gene to be regulated appears very conserved. This we conclude from the experiments of Mitsialis & Kafatos (1985) who inserted two chorion genes of the lepidopteran *Bombyx mori* into the genome of the dipteran *Drosophila melanogaster*. The *cis*-elements of the *Bombyx* genes reacted to the *trans*-regulatory signals of *Drosophila* by proper transcription in the **follicle** cells of **females** at the right **time**, even though the two species have evolved separately for some 240 million years.

The importance of the cell type for the regulation of the differentiation genes was recently demonstrated by DiBenedetto *et al.* (1987) for the male-specific transcript *mst 316*. This RNA is only transcribed in the paragonia which are accessory glands of the male genital duct; females do not have a corresponding tissue. As expected, *mst 316* is also present in X/X; $tra\text{-}2^{ts}/tra\text{-}2^{ts}$ pseudomales raised at 29°C.

When these pseudomales were shifted to 16°C where the product of *tra-2*^{ts} is functional, *mst 316* nevertheless continued being synthesized. This is in contrast to what is observed for the YP genes in the fat body, a tissue that is common to both sexes. In this latter case, the activity of the YP genes depends on a functional product of *tra-2*. Once a sex-specific tissue, such as the paragonia, is differentiated, however, its synthetic machinery might become independent of the state of the sex-determining genes. These would then only be required to produce the sex-specific tissue.

Parallels to other organisms

When we compare our Fig. 1 with the genetic pathway of sex determination of *Caenorhabditis* as described by Hodgkin in this volume, we notice a surprising similarity between the nematode and *Drosophila*. Both organisms use the X:A ratio as the primary signal to regulate a key gene that acts at the top of a genetic cascade consisting of a few subordinate control genes. Mutations in these control genes show that the purpose of the cascade is to achieve differential activity of the last gene, *tra-1* in the case of *C. elegans*, *dsx* in the case of *Drosophila* and that the sex of the organism ultimately depends on the state of activity at this locus. It is remarkable that we find these similarities in those two higher organisms whose mechanism of sex determination has been most thoroughly analysed. The parallels may be the result of pure coincidence; after all, the phylogenetic distance between the unsegmented nematode and the metameric fly is formidable. But it is also possible that the parallels have arisen because the same genetic strategy is used by a variety of organisms and by remote systematic groups. The basic scheme involving a *signal*, a *key gene*, and a few *subordinate control genes*, can be detected in many more organisms, including other insects, mammals, and even plants (Nöthiger & Steinmann-Zwicky, 1985*b*, 1987).

At the phenomenological level, a large variety of sex-determining mechanisms appears to have evolved. Very different systems, however, which include sex determination by a chromosomal signal (Y chromosome, X:A ratio), dominant male-determining factors, dominant female-determining factors, maternal or even environmental sex determination can easily be explained by assuming simple allelic variations in one or two of the control elements (Nöthiger & Steinmann-Zwicky, 1985*b*, 1987). Two examples illustrate this point. (1) The mutation *tra-2*^{ts} of *Drosophila* shows how a system with genetic sex determination can evolve into one with environmental sex determination as a consequence of a temperature-sensitive mutation in a single control gene (for the sake of the argument, we will ignore the fact that such males are sterile). (2) For *Caenorhabiditis elegans*, Hodgkin (1983) has turned the natural system with chromosomal sex determination into one that functions with a single allelic difference at the last gene in the cascade, *tra-1*. Males are homozygous for a recessive null allele, *tra-1*^{n}; females are heterozygous for *tra-1*^{n} and a dominant constitutive allele, *tra-1*^{c}. In this new 'species', mutations in control genes that act upstream of *tra-1* will have no consequence and will therefore remain undetectable in genetic tests. No geneticist would suspect the close relationship to the original *C. elegans*. The two examples just described show how easy it is to evolve new sex-determining mechanisms that for the superficial observer appear to be very different.

The more we know about the genetic control of sex determination of an organism, the better it fits into our general scheme. *Musca domestica*, the common housefly, shows more and more parallels to *Drosophila*. Mutations are being discovered that are functionally homologous to *da* and *Dk* and to the constitutive mutation *Sxl*^{M} (Inoue & Hiroyoshi, 1986).

The homology to which we here refer applies to the genetic structure of the system, and not necessarily to the sequence of bases in the DNA. Although we expect to find homologous sequences in closely related species, more distant taxonomic groups may use different RNAs or proteins to control their sexual pathway. But the common structure would be a signal, a key gene to monitor the signal and subordinate control genes to reach the differentiation genes. In a comparison to human languages, we would say that the syntactic rules and the meaning of the sentences dictating the sexual pathway are the same in different organisms, but the sequence of letters may be very different:

I tell you: turn the switch on and make a female!
Ich sage euch: dreht den Schalter an und macht ein Weibchen!

There is not much sequence homology that a molecular biologist can detect between the English and the German sentence, and he would fail to recognize that they are structurally and functionally identical.

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