with respect to the magnetic field. There is an ecological advantage of preferably relying on celestial rotation for the basic direction. When innate information has to be transformed into an actual compass course, it is important that this results in a particular geographical direction. 'Away from the centre of rotation' always indicates true (geographical) south, whereas the local magnetic conditions may vary. Really strong magnetic anomalies are fairly rare; however, at higher latitudes, birds regularly meet large magnetic declinations (large deviations between magnetic and geographical north) and the most rapid changes in declination. Using celestial rotation as reference will thus help migrants to cope with unpredictable local magnetic conditions. It ensures that the magnetic course as well as the stellar course indicates the correct route when the birds start their migration. Indeed, experimental evidence so far indicates that celestial rotation dominates over information from the magnetic field when the migration course is first established at higher latitudes[3–7]. Later, during migration itself, when the birds have reached regions where magnetic declination is smaller and changes less rapidly, the magnetic field gains control[15–17]. We can only speculate why the population-specific deviation from due south is apparently encoded with respect to only the magnetic field. The direction derived from celestial rotation is a mental construct based on a rather complex integration of visual stimuli, whereas the magnetic field lines provide a stimulus that can be perceived directly. Possibly, it is easier to define angular deviations with respect to such a stimulus. The usefulness of the magnetic field for indicating angular differences is independent of the local relationship between magnetic and geographical north.

Another point emerges from our data: none of the groups showed the change in direction normally observed in the garden warbler at the end of September, when the free-flying birds leave Iberia for their African winter quarters. This is perhaps not surprising in the experimental group with their southerly tendencies, but the control group, after preferring a seasonally appropriate course during the first part of migration, might have been expected to change to a south-southeasterly heading. In previous studies with garden warblers, spontaneous changes in direction have been observed only when the birds were tested in the presence of suitable magnetic information[12,18], whereas birds tested under stars alone failed to show such a change[9]. Apparently, the population-specific course of the second part of migration also requires magnetic information. This suggests a transfer process analogous to that described for establishing the first direction. The behaviour of our control birds during October and November indicates that this process did not take place during the premigratory period when our test birds experienced celestial cues and the magnetic field together, but apparently occurs later, possibly shortly before the second part of migration begins. At that time, however, none of our test groups had access to magnetic information in the presence of stars. The increase in scatter observed in both groups might indicate a certain confusion caused by a lack of appropriate directional information. □

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# The dosage compensation system of *Drosophila* is co-opted by newly evolved X chromosomes

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**In species where males and females differ in number of sex chromosomes, the expression of sex-linked genes is equalized by a process known as dosage compensation. In *Drosophila melanogaster*, dosage compensation is mediated by the binding of the products of the *male-specific lethal* (*msl*) genes to the single male X chromosome. Here we report that the sex- and chromosome-specific binding of three of the *msl* proteins (MSLs) occurs in other drosophilid species, spanning four genera. Moreover, we show that MSL binding correlates with the evolution of the sex chromosomes: in species that have acquired a second X chromosome arm because of an X-autosome translocation, we observe binding of the MSLs to the 'new' (previously autosomal) arm of the X chromosome, only when its homologue has degenerated. Moreover, in *Drosophila miranda*, a Y-autosome translocation has produced a new X chromosome (called neo-X), only some regions of which are dosage compensated. In this neo-X chromosome, the pattern of MSL binding correlates with the known pattern of dosage compensation.**

Dosage compensation arises to deal with aneuploidy created by the degeneration of a sex-determining chromosome that occurs when recombination with its homologue is restricted[1,2]. The evolutionary relationships of dosage compensation systems with the mechanisms that control sex are complex and poorly understood. Sex determination evolves rapidly; considering just dipterans, XX–X0, XX–XY, ZZ–ZW and more complicated systems involving several pairs of chromosomes have been described[3]. Two factors contribute to this rapid evolution: (1) regulatory changes: if a new gene takes control of the sex-determination pathway, the chromosome on which it is found becomes the sex-determining chromosome; and (2) chromosomal changes: for example, in XX–X0 organisms, a translocation between the X chromosome and an autosome leads to restriction of the homologue, non-translocated autosome, to males, as a new Y chromosome. The occurrence of either of these types of changes will eventually lead to the degeneration of the new sex-limited chromosomes, thereby generating a need for dosage compensation of their homologues. Thus, is the pre-existing dosage compensation machinery that functions on one chromosome recruited to fulfil the requirements created by a new chromosome degeneration? *A priori* there is no reason to expect such a recruitment, because dosage compensation is acquired on a gene-by-gene basis[1]. Potentially, each gene could solve the problem in a different way. New mechanisms of dosage compensation could therefore arise to cope with the consequences of sex chromosome changes.

We have used a comparative approach to address this question

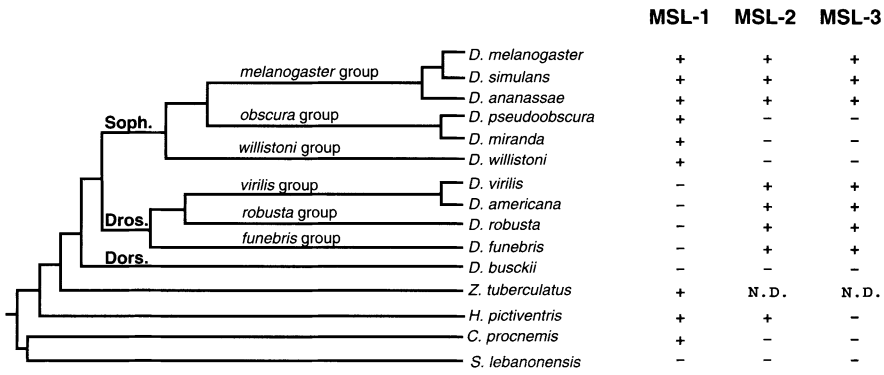


FIG. 1 Phylogenetic relationships among the species studied (based on refs 8, 11) and summary of results using the anti-MSL antibodies. The subgenera and groups are detailed for the *Drosophila* genus species (subgenera: Soph., *Sophophora*; Dros., *Drosophila*; Dors, *Dorsilopha*). Genera other than *Drosophila*: *Z.*, *Zaprionus*; *H.*, *Hirtodrosophila*; *C*, *Chymomyza*; *S*, *Scaptodrosophila*. +, male-specific staining in the arm or arms of the X chromosome; –, absence of sex-and chromosome-specific staining. MSL-1, MSL-2 and MSL-3 refer to the proteins against which the antibodies were raised (see Methods). N.D., not determined.

experimentally. Initially, we searched in other drosophilid species for the system first characterized in *Drosophila melanogaster*. In *D. melanogaster*, males are the heterogametic sex. Dosage compensation is mediated, for nearly all X-linked genes, by specific binding to the male X chromosome of the products of at least four genes, collectively named *male-specific lethals*: *msl-1*, *msl-2*, *msl-3* and *maleless* (*mle*). Binding of the MSL proteins increases the transcriptional activity of the genes on the male X chromosome to a level equal to that of the genes on both female X chromosomes[4]. Antibodies directed against the MSL-1, MSL-2 and MSL-3 proteins have been used for immunostaining in 14 drosophilid species. In 12 of these species, male X chromosome-specific staining by at least one of these antibodies is observed (Figs 1, 2). As in *D. melanogaster*, where female-specific repression of the production of MSL-2 protein prevents the function of the *msl*-based system[5–7], staining in females has not been observed in any of these 14 species. The male- and chromosome-specific binding of the MSL proteins provides strong evidence for the functional conservation of this system of dosage compensation in all of these species. Interestingly, staining is seen in both *Chymomyza* and *Hirtodrosophila*, among the more distant relatives of *Drosophila* within the *Drosophilinae* subfamily[8]. The *msl*-based system of dosage compensation is therefore much older than the *Drosophila* genus; the *Chymomyza–Drosophila* split is estimated to have occurred at least 55 million years ago[9–11].

Using the anti-MSL antibodies, we have been able to examine whether the MSL-based system is responsible for dosage compensation in situations where structural changes in the sex chromosomes have occurred. Occasionally during the diversification of the *Drosophila* genus, X-autosome translocations have been fixed, generating new X chromosome arms (Fig. 3). In *D. americana*, the homologue of the translocated arm has not yet degenerated, therefore it is not expected that dosage compensation will be required in the new X chromosome arm. In this species, MSL binding is observed in a single chromosomal arm (Fig. 4*a*, *b*). In three independent cases, where the homologue of the translocated (ex-autosomal) arm is fully degenerated, binding of the MSL proteins to the new arm of the X chromosome is observed (*D. pseudoobscura*, *D. willistoni* and *D. robusta*; see Fig. 4*c–h*). The number of sites stained is similar in both arms and is comparable

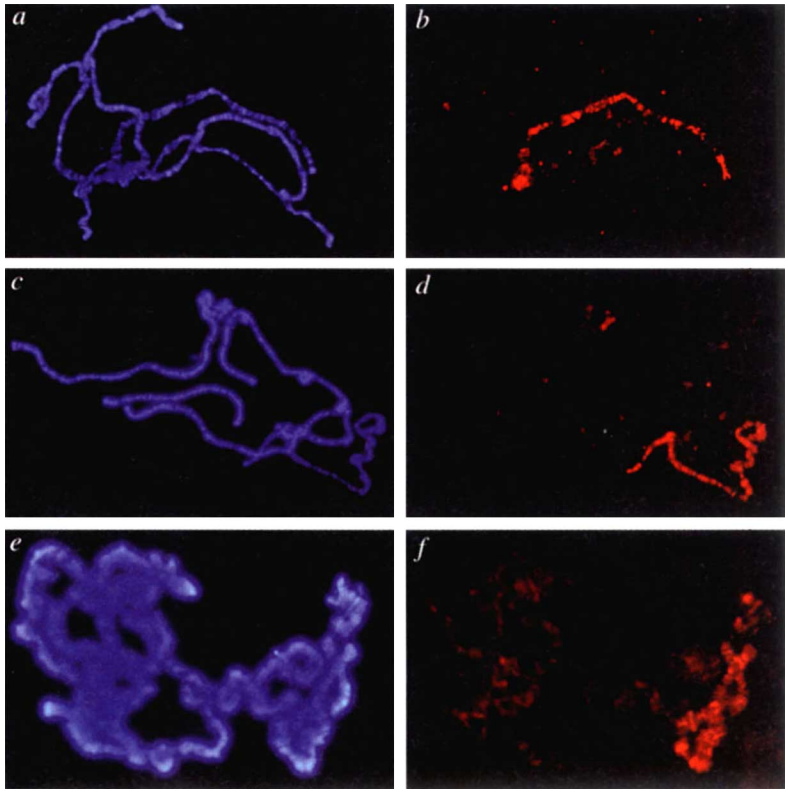


FIG. 2 Immunostaining in males of several drosophilid species. Left panels, DAPI staining; right panels, anti-MSL staining. *a*, *b*, *D. virilis* (the right panel shows the anti-MSL-3 antibody signal). *c*, *d*, *Zaprionus tuberculatus* (anti-MSL-1). *e*, *f*, *Chymomyza procnemis* (anti-MSL-1).

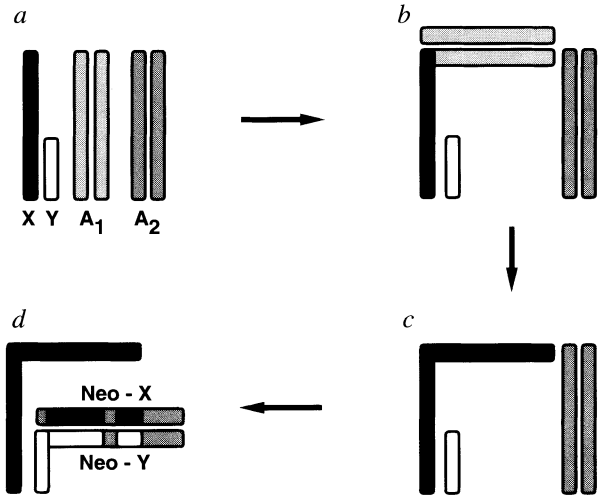


FIG. 3 Sex chromosome evolution in selected species of the *Drosophila* genus. Arrows refer to the sequential steps in chromosomal evolution; no phylogenetic relationship among species is implied. *a*, Simplified male karyotype of most *Drosophila* species. Only three of the five pairs of chromosomes are shown. The X chromosome is dosage compensated (black), whereas the Y chromosome is inactive (white). $A_1$ and $A_2$ are two pairs of autosomes. *b*, Karyotype found when an X-autosome translocation is fixed and the non-translocated arm has not yet degenerated (for example, *D. americana*). *c*, Karyotype after the homologue of the translocated chromosome has degenerated (for example, *D. willistoni*, *D. pseudoobscura* and *D. robusta*). *d*, In *D. miranda*, a recent (at most two million years old[9]) Y-autosome translocation has occurred, in addition to the X-autosome translocation characteristic of *D. pseudoobscura* and its relatives[16]. The autosomal arm that has been fused to the Y chromosome (neo-Y) is partially degenerated (white patches) and its homologue (neo-X) is partially dosage compensated (shown as black patches)[13,14].

to that observed in *D. melanogaster*. Previous experiments demonstrated that in *D. willistoni* and *D. pseudoobscura*, both arms of the X chromosome (shown in black) were fully compensated[12]. In *D. miranda*, a recent Y-autosome translocation has occurred (Fig. 3), in addition to the X-autosome translocation also observed in its sibling *D. pseudoobscura*. The neo-Y chromosome is not fully degenerated, and some regions of its homologue (neo-X, also known as $X_2$) do not show increased transcriptional activity[13,14]. In the neo-X chromosome there are fewer sites showing MSL binding than in the arms of the X chromosome, and the zones with binding correlate with those zones known to be dosage compensated (Fig. 4*i*–*m*). These results are expected if, as suggested for *D. melanogaster*[4], the *msl*s are the direct mediators of dosage compensation in all these species.

Our results have several implications. First, they show that the *msl*-based system is older than the *Drosophila* genus. Second, because this system can be co-opted to new chromosomes during evolution, it may be older than the degeneration process that causes a requirement for dosage compensation in new chromosomal arms. Therefore, the *msl*-based system may operate in diverse organisms, even if their sex determination systems are different. Third, in four independent cases, the solution to the

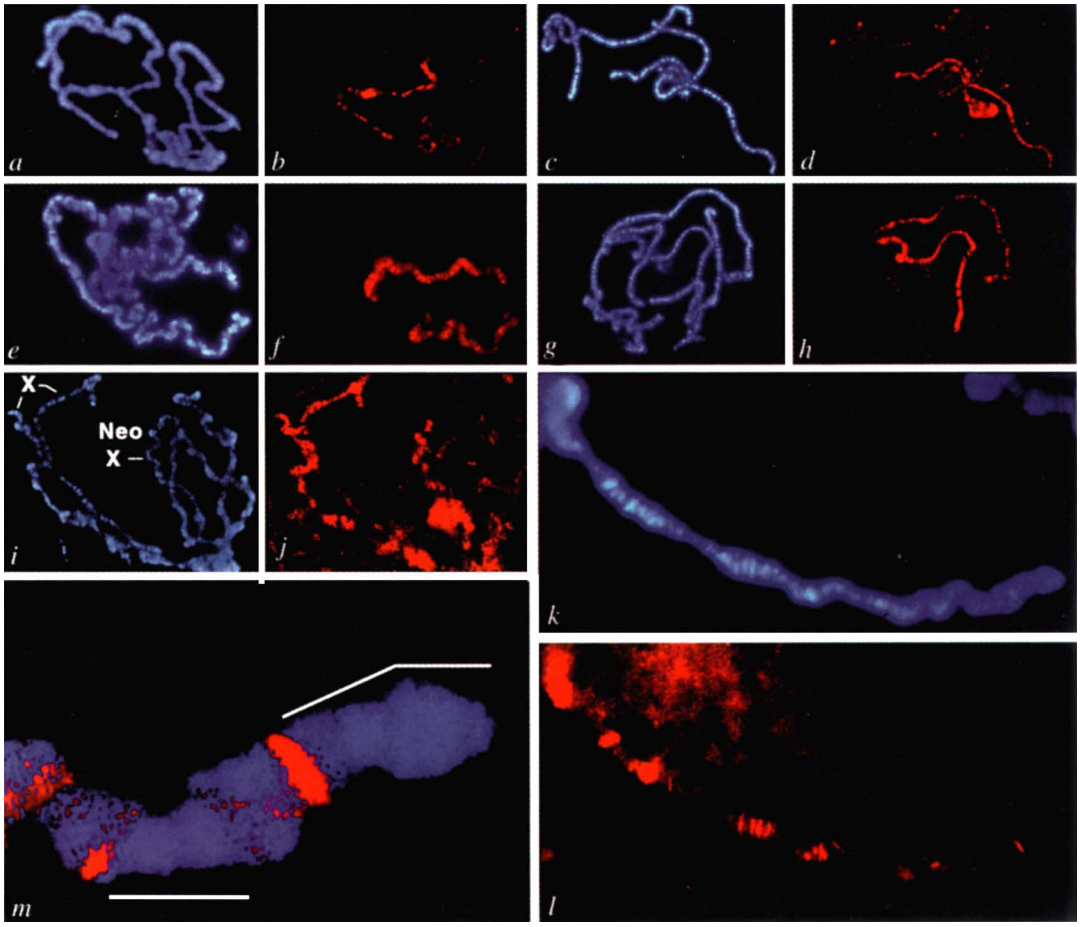


FIG. 4 Immunostainings showing the correlation between degeneration of a chromosome and acquisition of MSL binding in its homologue. *a*, *c*, *e*, *g*, *i*, *k*, DAPI staining; *b*, *d*, *f*, *h*, *j*, *l*, antibody staining. *a*, *b*, *D. americana* (anti-MSL-3 antibody); binding is present only in one of the arms of the X chromosome. *c*, *d*, *D. pseudoobscura* (anti-MSL-1). *e*, *f*, *D. willistoni* (anti-MSL-1). *g*, *h*, *D. robusta* (anti-MSL-3). In *c*–*h*, where the non-translocated homologue has degenerated, both arms show binding. *i*–*m*, *D. miranda* (anti-MSL-1 antibody). *i*, *j*, General view. Three chromosomal arms, both arms of the X plus the neo-X, are stained. *k*, *l*, Close-up of the neo-X chromosome of *D. miranda*. The number of bands, especially near the telomeric end, is not as abundant as usually observed in the X chromosomes. *m*, Anti-MSL-1 staining (red) superimposed on the DAPI staining (blue) in the tip of the neo-X chromosome of *D. miranda*. The white lines correspond to the zones determined to be non-dosage compensated[14].

problem of sex chromosome degeneration has been to extend the *msl*-based mechanism. This has occurred despite the fact that, on an evolutionary time scale, the requirements for compensation appear gradually, gene by gene. These results suggest that the extant compensatory system is adopted when new requirements for dosage compensation arise. The constraints on developing new dosage compensation mechanisms probably derive from the difficulty in evolving systems able sex-specifically to regulate functionally unrelated genes, which have in common only the fact that they reside on the same chromosome. Taking all these considerations together, we expect to find identical mechanisms functioning in phylogenetically very distant species.

*Note added in proof*: Histone H4 acetylated at lysine residue 16, known to be implicated in the hypertranscription mechanism in *D. melanogaster*, is also enriched in the newly evolved X chromosomes of *D. pseudoobscura* and *D. miranda*[17]. □

## Methods

**Antibodies.** We have used rat polyclonal antibodies against three of the MSLs (MSL-1, amino acids 618–939; MSL-2, 534–669 (ref. 5); and MSL-3, 324–513 (ref. 15)). These antibodies were selected on the basis of results of preliminary experiments using *Drosophila pseudoobscura*, *D. virilis* and *Chymomyza procnemis*. Only these antibodies stained the male X chromosomes of at least one of these species. Six other antibodies, including two raised against MLE, gave negative results. The results summarized in Fig. 1 are based on at least two preparations for each combination of antibody, sex and species. The negative assessments for *Scaptodrosophila lebanonensis* and *Drosophila busckii* males are based on at least 5 chromosome preparations per antibody. The number of epitopes recognized in distant species is probably small because: (1) none of the antibodies is positive in all the species; (2) positive signals do not strictly correlate with the phylogenetic proximity to *D. melanogaster*; and (3) signal intensity in other species is lower than in *D. melanogaster*. Therefore, the simplest explanation for the failure to observe binding in those two species is that they have lost the conserved epitope(s) recognized by our antibodies.

**Immunostainings.** Third instar larvae were sexed according to the size of their gonads. Sex was verified by observation of polytene chromosomes from the salivary glands. Under phase contrast optics, males showed one to three pale arms, depending on the species. Moreover, in some cases the male X chromosome had a peculiar shape, was shorter or seemed diffuse when compared with the autosomes. When possible, it was further determined that the stained chromosome(s) corresponded to the X chromosome(s) by comparison with the available chromosome maps. Immunostainings were performed as described in ref. 5. Antibodies were generally used at the same dilution routinely chosen for *D. melanogaster* (1:50). Although at this concentration the intensity of staining observed in the other species was substantially lower than in *D. melanogaster*, the male X chromosome was easily visualized in every preparation of most species. Only in *D. pseudoobscura*, *D. miranda* and *Hirtodrosophila pictiventris* was the fluorescence level so low that in some slides the X chromosome was not easily distinguishable. In these species, higher concentrations (up to 1:20) were used. DAPI was used to stain the chromosomes for localization and study under fluorescence optics.

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# Functional neuroanatomy of human rapid-eye-movement sleep and dreaming

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**RAPID-EYE-MOVEMENT (REM) sleep is associated with intense neuronal activity, ocular saccades, muscular atonia and dreaming[1,2]. The function of REM sleep remains elusive and its neural correlates have not been characterized precisely in man. Here we use positron emission tomography and statistical parametric mapping to study the brain state associated with REM sleep in humans. We report a group study of seven subjects who maintained steady REM sleep during brain scanning and recalled dreams upon awakening. The results show that regional cerebral blood flow is positively correlated with REM sleep in pontine tegmentum, left thalamus, both amygdaloid complexes, anterior cingulate cortex and right parietal operculum. Negative correlations between regional cerebral blood flow and REM sleep are observed bilaterally, in a vast area of dorsolateral prefrontal cortex, in parietal cortex (supramarginal gyrus) as well as in posterior cingulate cortex and precuneus. Given the role of the amygdaloid complexes in the acquisition of emotionally influenced memories, the pattern of activation in the amygdala and the cortical areas provides a biological basis for the processing of some types of memory during REM sleep.**

Thirty young, healthy, right-handed male subjects (mean age, 22.5 years; range, 20–25) participated in this study, which took place over 3 polygraphically monitored nights spent on a scanner couch, at one-week intervals. Polygraphic recordings included electroencephalogram (recorded between electrode pairs C3–A2 and C4–A1), EOG (electro-oculogram) and chin EMG (electromyogram), and were scored using the criteria of ref. 3. Subjects were asked not to sleep during the night preceding the second and third test nights to ensure optimal sleep stability despite difficult experimental conditions (head stabilized using a face mask secured to the scanner head holder and left arm cannulated and immobilized). Subjects were selected if they could maintain two periods of slow-wave sleep and of REM sleep (20 min each) during the first two test nights. Nineteen subjects were finally selected.

During the third test night, regional cerebral blood flow (rCBF) distribution was recorded as an index of neuronal activity using six slow intravenous infusions of $H_2{}^{15}O$ scheduled during the following states: wakefulness, slow-wave sleep, slow-wave sleep, REM sleep, REM sleep, wakefulness. This order was imposed by the physiological preponderance of slow-wave sleep early in the night and of REM sleep in the early morning. During sleep, scans were done when polysomnography showed characteristic sleep patterns (slow-wave or REM sleep) that remained stable over the 5-min period necessary for water production. After each sleep scan, the subjects were woken up and asked to describe what was in their minds. Dream reports were scored following the classification system of ref. 4. Four subjects were studied during a complete set of states (2 wakeful, 2 slow-wave, 2 REM sleep states); another group of three subjects was scanned during 2 wakeful and 2 REM sleep states but changed their sleep stage during the injections in the slow-wave sleep state. All subjects recalled a dream after awakening from REM sleep. The dream contents included various settings, characters, objects and activities. A final group of four subjects were scanned during 2 wakeful and 2 slow-wave sleep