Another way of looking at the enigma of sex determination in *Ellobius lutescens*

Any heuristic can be treacherous, but a Darwinian explanation is the first I would seek in explaining a biological enigma.

– J. Lederberg[1]

A number of hypotheses have been proposed over the years to account for sex determination in the vole *Ellobius lutescens* (Arvicolinae, Rodentia, Mammalia) (reviewed in ref. 2). The chromosomes of the males of this species are identical to those of the females in number and morphology, with the same odd number ($2n$ = 17) being present in both sexes[3]. The odd chromosome is the X chromosome, and it constitutes approximately 5% of the haploid genome. It therefore appears that the X chromosome is of the standard ('original') type found in most mammals[4]. Embryonic mortality is high[5], as one would expect when both parents are monosomic for the X chromosome and if embryos disomic for the X do not survive. The X remains unpaired at meiosis[6]. There is no evidence of a Y chromosome, either in the soma or in the germline. The possibility of *Sry* (*S*ex determining *r*egion of the *Y*) being present as a cryptic translocation in another chromosome has recently been ruled out by the finding that *Sry* sequences are not detectable in *E. lutescens*[7]. On the other hand, in *Ellobius fuscocapillus*, a related species with a standard XX female–XY male sex-chromosome system, males are *Sry*-positive and females are not[7]. Thus, there has evolved a system in which both sexes are XO, sex determination occurs without *Sry*, and meiosis and spermatogenesis are maintained without a Y chromosome. These cytogenetic features, unexpected in a mammal, have prompted some authors to describe sex determination in this species as enigmatic. Unravelling the complexity of this system – and that of other exceptional systems[8] – is a prerequisite for identification of the common, and therefore core, elements of mammalian sex-determining mechanisms.

There are aspects of the *E. lutescens* chromosome system that are reminiscent of the mechanisms that lead to sex determination in the fungus gnat *Sciara coprophila*[9] and the coccids *Pseudococcus* (= *Planococcus*) *citri*[10,11] and *Pulvinaria hydrangeae*[12] (see (b) and (c), below). These apparent similarities prompt the hypothesis, described below, that regulation of sex determination in *E. lutescens* may occur through genomic imprinting. I propose that imprinting leads to two types of embryos: those in which there is a reduction in the effective copy number of particular sex determination genes and others in which there is no such reduction. The two types of embryos would differentiate into the two sexes.

The hypothesis that imprinting may have an essential role in mammalian sex determination has been stated before[13–16], especially in relation to the evolution of X chromosome inactivation and its functions[13–17], but it may be useful nevertheless to list the observations upon which it rests:

(a) In a large number of animal species, embryos which develop into males differ from those that develop into females in the dosage of particular genes. In mammals, dose-dependent effects on sex determination[13,14] are known for at least three

genes[18–20].

(b) A reduction in effective gene dosage, from two to one (or one to none), is a key consequence of genomic imprinting[21]. In several insects, imprinting is closely related to sex determination. This relationship is clearly seen in the three insect species in which imprinting was first recognized and experimentally investigated [*S. coprophila*[9]; *Pseudaulacaspis pentagona*[22]; *P. citri*[10,11]]. In each of these species, all zygotes are chromosomally identical. However, as a consequence of genomic imprinting, which manifests itself as chromosome inactivation
or elimination, a difference emerges early in embryonic development
in the actual or effective copy number of particular chromosomes between embryos that develop into males and those that develop into females[23].

(c) In sexually-reproducing coccids, imprinting apparently affects genes from only one parent, those from the father[10,22,24]. Certain parthenoge-netic coccids (eg. *Pulvinaria hydrangeae*)[12,25,26] occasionally reproduce sexually. In such insects, transition from the parthenogenetic to the sexual phase occurs by the imprinting and inactivation of one of two sets of maternal chromosomes, but in the immediately following (sexual) generation, the set imprinted is paternal[12,25,26]. Among eutherian mammals, some autosomal genes are maternally imprinted whereas others are paternally imprinted[27]. Eutherian X inactivation, which is random in the embryo proper but paternal in the extra-embryonic tissues, is considered here, as in previous publications[17,21,23], as an outcome of imprinting because of the closeness of its evolutionary connection to the marsupial system of paternal X inactivation[28,29] and because, whether random or paternal, its proximate function is the same: hemizygous expression of X-linked genes in females. Random X inactivation is expected to have been favoured during eutherian evolution because of the advantages it confers over paternal X inactivation, the presumed ancestral condition[17].

(d) In humans and mice, imprinted X-chromosomal domains are recognizable as heterochromatic regions, but the sparse distribution of imprinted loci on autosomes does not permit a similar visual recognition. Thus, if imprinting affects only a small number of genes, the chromatin domains which include such genes may be too small to appear heterochromatic under the microscope.

When, as in *E. lutescens*, chromosome number and morphology do not differ in the two sexes, how does one distinguish between imprinting and the other regulatory processes that could result in sex determination? Among *E. lutescens* autosomes, one would expect to see differential regulation of homologous sex determination genes[21]. Such regulation may be accompanied by a parent-of-origin effect such as that seen among X-linked genes in marsupials[28,29] and in the *IGF2/H19* complex[27] in humans, or be without it, as among X-linked genes in eutherians. On the other hand, in case of genes on the single X chromosome of *E. lutescens*, such silencing could be parent-of-origin dependent, but occurring in one sex and not in the other. The effective dosage of X-linked genes so silenced would then become reduced from one to zero. In embryos developing into females, one would expect to find genes concerned with maleness to be imprinted, and in those developing into males, genes for femaleness are expected to be among those imprinted. On the basis of what is known in coccid genetic systems, one other expectation may be stated: the sex

ratio in *E. lutescens* is likely to be variable and subject to environmental influences, especially those acting on the mother.

There may be difficulties associated with testing this hypothesis in *E. lutescens*. For instance, genetic variation in this rodent is poorly documented. Identification of imprinted genes by pedigree analysis is hampered by the fact that these animals breed poorly in captivity. CpG methylation could be an alternative criterion for identifying imprinted genes. Two genes, *Dax1* and *Sox9*, show evidence of dosage-dependent roles in mouse sex determination[18,19]. *DAX1* and *SOX9* have similar effects on human sex determination[19,20]. *DAX1*/*Dax1* is of particular interest because it is X-linked, and subject to inactivation. Its dose-dependent regulation can therefore be regarded as a product of X-

chromosome imprinting[17]. When abnormally present in two copies in XY individuals, it overrides the effects of *SRY*/*Sry* and causes sex reversal. *E. lutescens* equivalents of these genes, and that of *Sox3* (ref. 30) – the closest known relative of *Sry*-, may be good candidates for studying whether there are such epigenetic differences between male and female voles.

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