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Review

Unusual Ways to Lose a Y Chromosome and Survive with Changed Autosomes: a Story of Mole Voles *Ellobius* (Mammalia, Rodentia)

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Abstract:

Species of mole voles *Ellobius* demonstrate a broad variation in sex chromosomes and autosomes, which is unique among mammals. In four species, a Y chromosome was lost, and X0 or XX sex chromosomes in both sexes were obtained. The key testis-determining *Sry* (*Sex-determining Region on Y*) gene is absent in these species, and the regulation of its target, the *Sox9* (*SRY -box 9)* gene, is questionable due to deletion in the key enhancer. In a single species, *E. fuscocapillus*, with routine XX-XY, the same deletion is present alongside fragments of *Sry* in the female genome. Presumably, a Y chromosome was lost twice in two phylogenetic lineages of mole rats; before the event, a few male-specific genes escaped on X chromosomes. Translocations of Y chromosome fragments were made independently, resulting in different changes in species without a Y chromosome and the presence of the Y-linked *Sry* gene in females of *E. fuscocapillus,* a species retaining the Y chromosome. One more exceptional phenomenon is high autosomal variability in *E. tancrei.* This species might be used as an exclusive model for studying meiotic mechanisms providing balanced gametes

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in complex heterozygous hybrids. Sterility is the only destiny for hybrids, whose parents carry Robertsonian translocations with partial homology. Contrary to that, *E. tancrei* possess different Robertsonian translocations and successfully overcome the hybrid incompatibility. Here, we overview the research to date of sex determination and meiosis in *Ellobius*.

Keywords
Sex determination; meiosis; sex chromosomes; Robertsonian translocations

1. Introduction

Gene interaction during early development determines the development of gonads, producing gametes in a distinct way in males and females. Meiosis is essential to provide a precise transmission of parental genetic information to the next generations, which is a base for effective reproduction and evolutionary success. The evolutionary plasticity of a primary switch of sex determination in vertebrates, such as genetic, epigenetic, temperature-dependence or other types of elusive nature, remains poorly understood [1-3]. Three modern infraclasses of mammals (monotremes, marsupials and placental) have significant differences in their sex chromosomes. Monotremes, the most diverged mammalian group, obtained multiple sex chromosomes — 10 elements in platypus males and 9 in echidna males [4, 5]. Marsupials and placental mammals separated from monotremes approx. 166 million years ago [6], and evolved with sex chromosomes XX-XY, the gene content and structure of sex chromosomes appeared to be different in the groups. Evolution of their heteromorphic sex chromosomes, as suggested due to molecular studies of a Y chromosome, started approximately 180 million years ago in an ancestor of Eutheria [7].

A Y chromosome evolution might be initiated by the emergence of a specific testis determining factor and decreasing recombination between originally homomorphic chromosomes. Subsequently, these chromosomes evolved into heteromorphic chromosomes associated with sex [8, 9]. The suppression of recombination developed as a result of numerous chromosome changes, mostly inversions, creating at least four evolutionary strata at sex chromosomes [10]. The schematic network of sex determination should include several components: a primary genetic signal, the key gene, which is a target to this primary signal, and a gene switching between two alternative programmes of development [11].

Sex determination is a strictly scheduled process when the fine-tuned gene expression levels are provided. In placental mammals the gene *SRY* (Sex-determining Region on Y) is a key factor that determines the formation of testes (the Testis Determining Factor, TDF) and locates on the Y chromosome [12-14]. The prime role of the *SRY* is to activate the *SOX9* (SRY -box 9), which should later (after 11.5 days post coitum (dpc) in mice) activate other genes in the male pathway, such as fibroblast growth factor 9 (*FGF9*) and fibroblast growth factor receptor 2 (*FGFR2*). The *FGF9* and *FGFR2* genes normally block *Wnt4* (Wnt family member 4) in males. In a case of down regulation of *FGF9*, Wnt4 blocks *SOX9* and starts the female pathway by activating β-catenin, forkhead box L2 (*FOXL2*) and others. The idea that female sex is programmed by default and a specific male-determining factor was originated to override it has prevailed since the *Sry* gene was described

(for review see [1]). However, the process of sex determination is apparently more complex. First, there is data on the epigenetic regulation of *Sry* expression and Sertoli cells differentiation, such as DNA methylation and histone modifications, for example, by the histone demethylase *JMJD1A* (Jumonji domain containing 1A) and others [15-17]. The ovarian development in mice starts later, at 12.5 dpc, and now it is clear that a 'default' mechanism is not efficient. The ovarian pathway is studied intensively, with a main accent on the *RSPO1* (R-spondin1) gene [18]. Whereas male (testes) development depends on a single pathway of *SOX9* regulation, the female (ovaries) development is controlled by at least two ways of *SOX9* suppression [19]. The intensive studies of signalling pathways involved in mammalian sex determination revealed a tremendous amount of regulation events, including epigenetic ones [3, 16, 17]. One of the most amazing features of sex determination is a possibility of sex reversal in adult mammals. That means a transformation of testes to ovaries and vice versa and entails a life-long struggle against an alternative sex determining pathway by the repression of several key genes [20, 21]. The early mammalian gonad contains bipotential precursor cells for steroid-secreting cells and supporting cells, which sustain and nourish germ cells. In male gonads, supporting cells can develop into Sertoli cells; in ovaries, to specific follicle (granulosa) cells. Sex determination and a reverse process both depend on the state of supportive cells, which should achieve a threshold in early development for getting 'proper' gonads, but even in adulthood, these supportive cells should keep a status quo. It was shown that in adult mice, an ablation of *Foxl2* in granulosa cells leads to the *Sox9* expression, which induces the transdifferentiation of granulosa cells to Sertoli cells [20]. In contrast, the deletion of the *Dmrt1* (doublesex and mab-3 related transcription factor 1) in testes results in the *Foxl2* upregulation and transformation of testicular Sertoli cells to ovarian granulosa cells [21]. *Sox9* and *Sox8* (SRY-box 8) together with *Dmrt1* protect the adult testis from male-to-female genetic reprogramming and complete degeneration [22].

The *Sry* gene is present in the marsupial male genome and probably has a function as a sex determination gene [23, 24]. There are also exceptions to the rule in placentals: several rodents have lost a Y chromosome and a primary sex determining gene *Sry*, such as several species of mole voles *Ellobius* [25] and spiny rats *Tokudaia* [26].

Our study was focused on the analysis of chromosome changes in mole-voles *Ellobius* from different points of view, with an emphasis on the meiosis and, especially, the behaviour of sex chromosomes, specific transmission of Robertsonian translocations between generations and variability of genes involved in sex determination and spermatogenesis. The primary hypothesis was that these phenomena are independent, and we tried to separately analyse the evolution of sex determination, genes and chromosomes, as well as autosomal variability in species with two X chromosomes in males and females. However, as we obtain more data, we have come to a conclusion that the evolution of sex chromosomes can probably play a leading role. Changes in sex chromosomes may enforce a genomic conflict, hybrid incompatibility, and, as an evolutionary output, generate reproductive barriers leading to species divergence [27]. Such ways of evolution might be favourable, even for such phylogenetic lineages, as mammalian infraclasses [28]. In this review, we aim to highlight the evolutionary history and uniqueness of sex determination without a Y chromosome and the elusiveness of primary sex determining genes in mole voles *Ellobius* alongside their special meiotic behaviour of sex chromosomes and variable autosomes.

2. Ellobius Species and Their Sex Chromosomes Variety

Mole voles *Ellobius* were described by Pallas in the XVIII century as *Mus talpinus* Pallas, 1770 [29], and remained poorly studied for over a hundred years, perhaps because of their secretive subterranean lifestyle. Fisher, in 1814, affiliated the species to a new genus *Ellobius* [30], which contained from two up to five species in a different taxonomy system [31]. R. Matthey, a famous Swiss cytogeneticist, revealed a unique mammalian odd diploid chromosomal number in *E. lutescens* (2n=17), and a single X chromosome in males and females [32]. An ordinary mammalian sex chromosome constitution XY♂ / XX♀ was recognized in the southern mole vole *E. fuscocapillus* (2n = 36) [33]. Three morphologically indistinguishable species lack a Y chromosome: the northern mole vole *E. talpinus* (diploid number 2n = 54, the total number of chromosome arms NF = 54), the eastern mole vole *E. tancrei* (2n = 54-30, NF = 56) and the Alai mole vole *E. alaicus* (2n = 52, NF = 56); all these species have two X chromosomes in females and males [33-36]. It was assumed that the whole or partial Y chromosome was translocated to the X chromosome or autosomes, but did not disappeared entirely, because males develop typical testes, and the sex ratio in all mole vole species is normal: approx. 50% of newborns are males, and 50% are females. The structures of autosomes and sex chromosomes were studied by chromosome painting of 4 species lacking Y chromosomes; the X chromosome is typical for rodents, and no parts of the Y chromosome were revealed [37, 38]. To date, only *E. fuscocapillus* was not studied by molecular cytogenetic methods. The chromosome painting data made it possible to reconstruct an ancestor karyotype for mole voles and evaluate changes in comparison with the Arvicolinae ancestor karyotype [37, 39].

The discovery of an odd chromosomal number in an *E. lutescens* karyotype arose a question of evolutionary history and functionality of a single X chromosome. How were a Y and the second X chromosome lost? How did the identical X chromosomes in males and females provide different sex determination? First, a hypothesis of fusions of two Xs in females and X with Y in males was declared [40, 41]. Nevertheless, studying meiosis [42-45] and applying competitive fluorescence in situ hybridization with genomic DNA probes (comparative genomic hybridization, CGH) disproved the hypothesis [46]. The 9th chromosome was proven as the X because the gene *G6pd* (glucose-6-phosphate dehydrogenase), typical for Xs, was detected on it [44]. Later, a comparative chromosome painting proved the assumption; the clear signal was distinguished on the 9th chromosome with X chromosome probes of several mammalian species, such as humans, mice, rats, *Microtus agrestis*, and *Mesocricetus auratus* [37], and no signal was detected for any autosomal probes. It is important that neither differences for male and female chromosomal sets, nor the signal for the Y chromosome probes were revealed. The same results were obtained for species with two X chromosomes in males and females of *E. talpinus* and *E. tancrei* [38]. These results proved the total loss of a Y chromosome, but chromosome painting has a restriction because a fluorescent signal might be unrecognizable in the case of a small fragment, whereas the screening for specific genes is more precise.

3. Tokudaia: by Rescuing Y Fragments, Obtain Duality

Another exceptional mammalian group, Ryukyu spiny rats (genus *Tokudaia*) are endemics of the Nansei Shoto archipelago, Japan. *T. osimensis* from Amami-Oshima Island and the recently described *T. tokunoshimensis* from Tokunoshima Island are closely related; both lost a Y

chromosome, although their diploid chromosome numbers are different: 2n=25, X0/X0 for *T. osimensis* and 2n=45, X0/X0 for *T. tokunoshimensis* [47, 48]. The differences (mainly centric fusions) were revealed by comparative chromosome painting [49]. An absence of the *Sry* gene was proved for *T. osimensis* [26, 50]. However, a small signal of a Y chromosome was revealed in *T. osimensis,* where a few genes were translocated to the X chromosome (*Zfy* and *Tspy*) [51] despite no differences for males and females being revealed by chromosomal painting and by comparative genomic hybridization analyses [52]. These species are endangered, so experiments on *T. osimensis* cells in vitro look very promising. The results revealed the dual potency for female somatic cells, which differentiated into male germline cells if the male reproductive niche occurred [53]. This experiment highlighted a tremendous significance of supportive cells in sex determination, which was discussed above. *T. muenninki* (Okinawa spiny rat) retains the Y chromosome unless both sex chromosomes are remarkably large due to fusions with autosomes [54].

4. In Search of a Primary Switch for Mole Voles

Loss of the *Sry* gene was demonstrated for *E. lutescens, E. talpinus* and *E. tancrei* along with revealing the high homology of the *Sry* sequence of *E. fuscocapillus* to humans and mice [25]. Later, W. Just and colleagues attempted to detect this gene in the genomic DNA of *E. lutescens* males using *Sry* probes of distinct species, changing hybridization conditions, etc., but got no specific signal [55]. Recently [56], we re-checked all five species (*E. alaicus* was unstudied before) and obtained unexpected results. In accordance with previous studies, we did not identify any *Sry*-similar sequences for males and females of *E. lutescens*, *E. talpinus*, *E. tancrei*, and, for the first time, *E. alaicus*. Surprisingly, the presence of a highly conservative HMG box *Sry* was revealed not only in males but also in females of *E. fuscocapillus*. Moreover, in different females, we detected variations in fragments of the HMG box from full (203 bp) to short ones (138 bp), and even an absence of it (in a single female). We suspected that the *Sry* gene exists in male and female genomes of *E. fuscocapillus* in multiple copies as a pseudogene; therefore, its functionality is questionable in this species.

Vogel et al. [57] hypothesized that sex determination in *Ellobius* species, which lost the *Sry* gene, might start by mutant alleles of some genes, which usually act downstream of the *Sry*. For studying the possible role of the genes, segregation analysis was developed. First, fragments of studied genes were amplified by PCR, cloned, and sequenced, and then polymorphic/biallelic markers were searched and screened in at least three generations of families of mole voles of no less than 20 specimens. The same strategy was used in mole voles for the main genes in the sex determination network: *SOX9*, *SF1* (Steroidogenic factor 1 or *Nr5a1 nuclear receptor subfamily 5 group A member 1*), *Sox3* (SRY-box 3), *Atrx* (alpha thalassemia/mental retardation syndrome X-linked), *Nr0b1* (nuclear receptor subfamily 0 group B member 1), *Ar* (androgen receptor), *Foxl2/Pisrt1* (Polled Intersex Syndrome Regulated Transcript 1 (Non-Protein Coding RNA)), and *Dmrt1* [25, 55, 58, 59]. No one demonstrated co-segregation of marker alleles with the sex of animals, therefore, a primary sex-determining function was excluded for all mentioned genes in *E. lutescens* and *E. tancrei*, i.e., species with X0 or XX sex chromosomes in males and females.

A precise gene expression regulation is essential for sex determination; an illustration is the *SOX9* gene, which is involved in numerous processes during development, as well as sex

determination. It is known that *Sry,* together with *SF1*, binds to the testis-specific enhancer core element (TESCO) of the *Sox9* gene to upregulate its expression [60]. Sox9 protein normally starts a genetic cascade in the bipotential somatic precursor cells, which develop into Sertoli cells, resulting in the development of testes. In case of the upregulation of *Sox9*, the foetal gonads develop as ovaries. Stability of the gene and an enhancer structure are needed because transcription factors should recognize specific binding sites in enhancers for regulation of the gene expression [61]. Nevertheless, in amazing *Ellobius* and *Tokudaia,* different deletions were found in the same testis-specific enhancer core element (TESCO) of *Sox9*. In *Tokudaia*, a deletion in TESCO was detected in XO species without the *Sry* gene and in the XY species with multiple *Sry* gene copies in *T. muenninki* [62]. In four studied species of mole voles, including *Sry*-positive species *E. fuscocapillus,* a 14-bp deletion was detected in the highly conserved module of the TESCO [63]. A deletion in the TESCO might lead to the gene *Sox9* upregulation unless a recent paper highlighted more appropriate enhancers for regulating the *Sox9* in early development of testis [64]. Nevertheless, the functionality of the sex determination gene network, starting with *Sry – SOX9* interactions, seemed doubtful in *E. fuscocapillus*.

It is possible that *E. fuscocapillus* restored their sex determination mechanisms by down-regulating the *SOX9* expression, as Bagheri-Fam et al. [63] hypothesized. The same method of solving the problem was proposed for *Tokudaia* species, in which numerous copies of the *Cbx2* gene might be involved in male sex determination, providing proper *SOX9* regulation [65]. No data for *Cbx2* was found in *Ellobius* yet, and this assumption needs to be checked.

Chandra [66] supposed that sex might be epigenetically determined in *E. lutescens* with a single X chromosome by transferring an imprinted X from mother to daughter and from father to son. However, analysis of specific microsatellite markers revealed their chaotic inheritance in three generations of mole voles, which was clearly demonstrated to be erroneous of the supposition [55]. The last hope was a whole genome study; a large group of researchers was involved in the project, the data were published and appeared to be rather discouraging [67]. Genomes of only two species, *E. lutescens* (male and female) and *E. talpinus* (female) were successfully sequenced and *de novo* assembled. Several known sex determination genes were recognized in male and female genomes of *E. lutescens*, and no new candidate sex-determining genes were revealed. To date, the enigma of sex determination in *E. lutescens* and *E. talpinus* remains unresolved.

5. The Loss of a Y Chromosome is Not a Deadlock

Although the whole-genome data did not answer the question of sex determining genes, it opened a way to study genes, which are usually located on mammalian Y chromosomes, in *Ellobius* species lacking this chromosome. Mulugeta et al. [67] detected the genes *Zfy1/2* (zinc finger protein Y-linked), *Eif2s3y* (eukaryotic translation initiation factor 2, subunit 3, structural gene Y-linked), *Ssty* (spermiogenesis specific transcript on the Y) in *E. lutescens* and *E. talpinus*, but *Usp9y* (ubiquitin specific peptidase 9 Y-linked) was determined in *E. lutescens* only. FISH demonstrated that the *Usp9y* and *Zfy* have been translocated to the X chromosome, so a Y was partly rescued. Although the genes *Eif2s3y, Zfy*, and *Ssty* were identified in males and females, the expression of these genes was detected in testes only. We sequenced fragments of the *Eif2s3y* of all *Ellobius* species, both males and females, and revealed, that an exonic part of *Eif2s3y* was demonstrated to identify up to 88% with the gene described for *T. muenninki,* a species with a Y

chromosome [56]. The non-exonic sequenced part was highly variable compared to *Ellobius* species but was identical in males and females for each species. This part of an *Eif2s3y* gene (206 bp) in all *Ellobius* species appeared to be a domesticated fragment of non-coding transposable short interspersed nuclear elements (SINEs) B2–B4 (http://www.repeatmasker.org).

In *E. fuscocapillus, Eif2s3y* exonic sequences were also identified in males and females. Along with *Sry* fragments in females, the presence of *Eif2s3y* proves the suggestion about the translocation of some Y-linked genes to the X chromosome in this species. The detection of the spermatogonial proliferation factor *Eif2s3y* in male and female genomes is especially interesting because it was shown that it is one of the two essential factors for spermatogenesis genes (the other one is the *Sry*, see [68]). With high probability, genes lost their primary function, but the data obtained in distinct species confirmed the independent duplications and translocations of some fragments from the ancestral Y to X chromosomes and, possibly, autosomes.

Such a method of compensation for Y degeneration was executed, not only in different *Ellobius* but also in *Tokudaia* species.

6. Dosage Compensation in a Case with a Single X Chromosome

The hypothesis of the necessity of dosage compensation for sex chromosomes declares the necessity to balance the gene products in somatic cells of males and females [69-71]. It is reliable, at least in part, in mammals with an XX-XY system, although its universal significance is controversial [72].

For *E. lutescens*, possessing a single X chromosome, 50% zygotic mortality was detected; zygotes without a sex chromosome, 2n = 16, 00 is lethal, and, unexpectedly, the regular mammals variant with 2n = 18, *XX* is lethal too [73]. The latter variant may be lethal due to insufficient X chromosome inactivation. The system of a sex chromosome dosage compensation is not well-studied to date, and it is suggested that the core event is the initiation of X-chromosome inactivation by nuclear long noncoding RNA (lncRNA) *Xist* (X inactive specific transcript) [74-76].

The structure of the *Xist* gene was studied in *Ellobius* and *Tokudaia*. In *Ellobius*, the studied fragment of the *Xist* gene had genus-specific changes, and a deletion, which is not present in *E. fuscocapillus* with heteromorphic sex chromosomes [55]. A deletion should lead to the functionality loss of the *Xist* and might be a source of lethality of embryos with 2n = 18, *XX* in *E. lutescens*. However, the question remains open on how males and females of three species survive with XX in both sexes.

Numerous mutations were detected in the *Xist* gene of the Ryukyu spiny rat *T. osimensis*, which should lead to the loss of a gene function in the XO/XO species. In another species, the Okinawa spiny rat, *T. muenninki*, with XX/XY and a neo-X obtained by fusion with an autosome, *Xist* RNAs were expressed in females [77]. Therefore, species with a single X chromosome do not need any mechanisms for X inactivation, their *Xist* gene remained in the genome, accumulated mutations and was degraded.

7. X0 and XX Sex Chromosomes in Meiosis

Meiosis, a crucial process of development, exhibits significant sexual dimorphism in features of gametogenesis in the two sexes, and the longevity and timing of meiosis differs in females and males. In females, meiotic prophase I starts in germ cells of the ovary during foetal development,

undergoes an arrest at the dictyotene stage and ends in adults; the last stages occur after fertilization. In males, meiotic prophase I starts in adult and occurs throughout most of their life, continuously providing gametes.

A diversity of sex chromosomes in *Ellobius* establishes distinct meiotic patterns. *E. fuscocapillus* develops a classical system for Eutherians, with a large submetacentric X chromosome and a small acrocentric Y. In the middle pachytene, Y achieved a complete synapsis with X and quickly underwent early complete desynapsis during the late pachytene-early diplotene [42, 45, 56]. Early desynapsis is uncommon for mammals and might be a sign of the lack of recombination, and further studies are needed. This feature resulted in an unusual 'typical' XX-XY system of *E. fuscocapillus.*

A single submetacentric X chromosome in males and females of *E. lutescens* is not similar to the X of *E. fuscocapillus* [42]. the X chromosome of *E. lutescens* is easy to distinguish as a single univalent in the meiotic prophase I. Using electron microscopy, we revealed that in the pachytene, the X-univalent became thicker and developed multiple axes and flexures ('hairpins'), and their structure was similar to synaptonemal complex. Such structures could be interpreted as possible sites for synapsis, but more evidence is needed for any conclusions. One or two enigmatic round bodies are usually located near the univalent. They are electron-dense, DAPI-positive and H2AFX-negative, and its functions are still unknown [56].

The functional differences were demonstrated for male and female XX chromosomes of *E. talpinus* and *E. tancrei*. These acrocentric XX chromosomes with identical G-band morphology underwent a complete synapsis during the pachytene I in females, but in males, XX synapsed and recombined only in the short telomeric regions [56, 78, 79]. The male XX chromosomes in *E. talpinus* and *E. tancrei* formed a typical sex body, which is similar to the XY body in other mammalian males, including *E. fuscocapillus*. One of the X axes has an electron-dense, SUMO1 (small ubiquitin-related modifier 1)- and DAPI-positive, H2AFX (H2A histone family member X)-negative round structure, which we previously called the dense nucleolar body [80], nucleolus-like body [42, 79] or chromatin body [78], respectively. Thus, the two X chromosomes in prophase I are not identical to each other. We suppose that asynapsis between isomorphic XX in males may occur due to asynchronous epigenetic chromatin changes [79]. This functional distinctness in males is presumably an evolutionarily new one, and it might be a start of neo-sex chromosomes in mole voles with two isomorphic X chromosomes.

8. Y Loss in Ellobius: How Many Times?

We hypothesized two independent losses of the Y chromosome for *E. lutescens, X0* and *E. talpinus-E. tancrei*, XX when revealing the specific behaviour of sex chromosomes in meiosis [56, 79, 81]. Mulugeta et al. [67] argued two independent losses of the Y chromosome for two studied species, *E. lutescens* and *E. talpinus*, and proposed a predisposition for the development of a new sex-determination system in the common ancestor of all mole voles. A study of all five species made the scheme more complex [56] (Figure 1).

The 14-bp deletion in TESCO is common for both subgenera of *Ellobius*; therefore, we started the reconstruction of their evolutionary history from this event (Figure 1). Unless the role of this enhancer in *Sox9* regulation during early gonadogenesis was overestimated [64], changes in the enhancer structure could be an accidentally discovered consequence of the genome

rearrangement, caused by an unknown crucial event. We assumed that after those genomic disturbances, species evolved in different ways due to their genome specificity. We suppose an independent loss of Y chromosomes in *E. lutescens* and in the subgenus *Ellobius* after the separation of two subgenera (Figure 1).

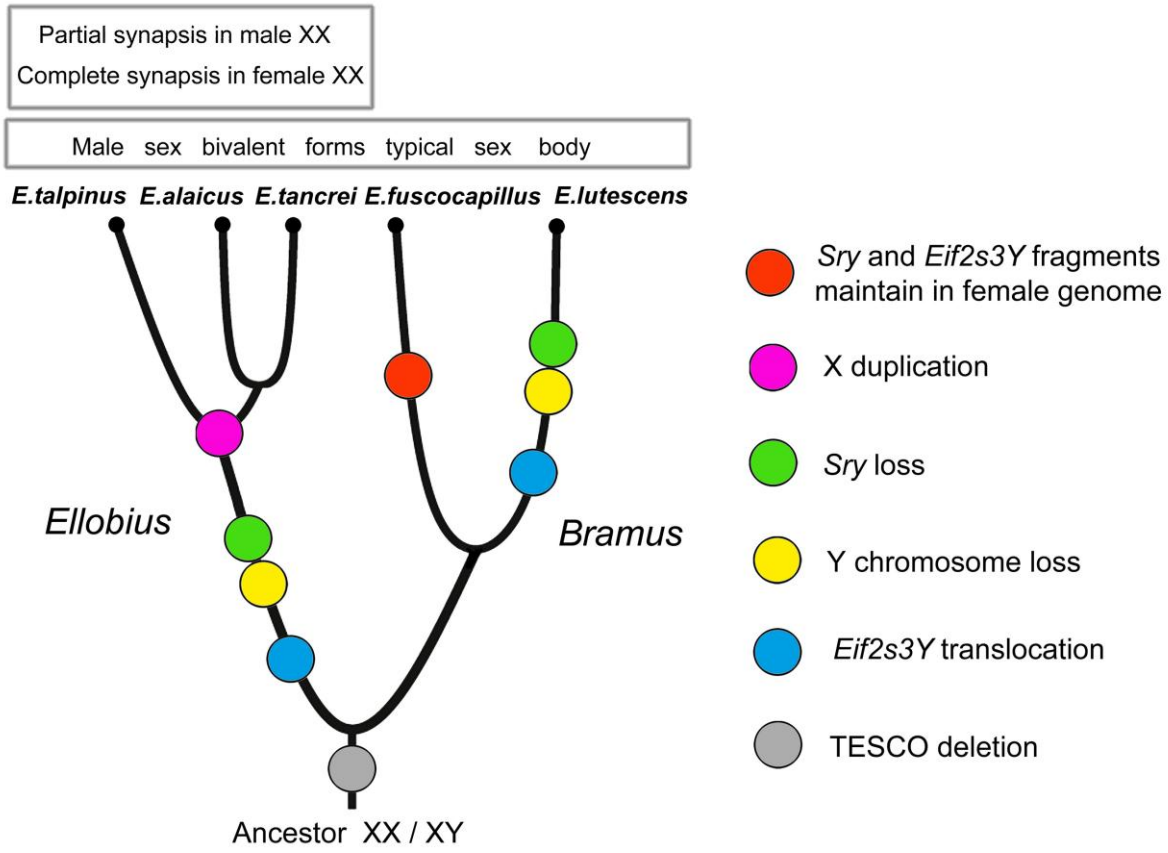

Figure 1 Evolution of sex chromosomes in mole voles *Ellobius*. Phylogenetic reconstruction is based on data of sex chromosomes and genes involved in sex determination and spermatogenesis.

Moreover, according to different changes in genes, species of two subgenera (*Bramus* and *Ellobius*) underwent distinct events of copying Y-linked genes and the loss of the entire Y chromosome. The presence of the *Sry* fragments in females of *E. fuscocapillus*, and the difference in structure of *Eif2s3y* between this species and *E. lutescens* revealed a high level of divergency in the subgenus *Bramus*. The comparison of structures of sequenced fragments of *Eif2s3y* and *Eif2s3x* genes alongside meiotic features led us to the conclusion that the X chromosome in *E. talpinus*, *E. tancrei* and *E. alaicus* was doubled after translocations of the Y-located genes to the X. Later, XX underwent a new heteromorphization, became functionally different in the central region, and restricted synapsis to telomeric areas in males (Figure 1).

Evolution of the X chromosomes in mole voles is different in subgenera too. In *E. fuscocapillus* and *E. lutescens,* the Xs are submetacentrics are different in size and G-band morphology. In subgenera *Ellobius,* the X chromosomes are large acrocentrics, undistinguishable by G-band in different species [42], and these chromosomes demonstrate functional distinctness in male and female meiosis [78, 79].

If a Y chromosome disappeared in some mole voles, might a single X in *E. lutescens* be lost too? This idea was discussed by W. Just (personal communication). He supposed a degeneration of the single X chromosome, which lost a partner for recombination, because a loss of recombination may lead to the accumulation of mutations. If mutations appeared to be deleterious, a mechanism of "Muller's ratchet" should eliminate species. However, even if non-deleterious mutations accumulate, they may inactivate X-linked genes and lead to extinction of the species.

The degeneration of the X chromosome might also be a start for the new cycle of sex chromosome evolution, when neo-sex chromosomes develop from autosomes. In *Ellobius* with two X chromosomes, we probably observe neo-sex chromosomes, which are morphologically identical, but functionally different.

9. Altered Autosomes and Meiotic Drive

Evolution of both sex chromosomes and autosomes may accompany or initiate speciation [82, 83]. In the case of sex chromosomes, as we tried to demonstrate above, the main point for a divergence is an emergence of different patterns in a meiotic behaviour of sex chromosomes. The same is true for autosomes. Inversions could lead to sterility; therefore, such chromosome changes are generally accepted as possible speciation mechanisms [82]. Mice are one of the most well-studied and fruitful groups for studying speciation by chromosome changes [84]. Many different fusions lead to different sets of metacentrics in karyotypes, along with different a combination of fused chromosomes. The same situation was found in *Ellobius tancrei*, one of the mole voles species with XX chromosomes in males and females [80, 85-89]. The most intriguing question is how numerous chromosomal changes could originate? A chain process, i.e., consequential fusions of acrocentrics, seems to be the simplest solution [90], but in the case of partial fusions, monobrachial homology is not an appropriate answer. The most promising is the idea that chromosome changes occur during gametogenesis; in such a case, we should conclude that the main mechanism of their origin must relate to meiosis. This hypothesis is rather problematic to prove because appropriate models and concepts of fixing changes should be developed. Robertsonian translocations were noticed as evolutionary neutral for a long time. Such a view may be true in a case of translocations, when hybrids are heterozygous by one translocation. In such a ‘simple’ case, a trivalent emerges in the pachytene stage, the meiosis ends with two types of gametes: with single metacentric or two acrocentrics. In the case of selective advantage for organisms with Rb translocation, it has a chance to spread; otherwise, it will keep as an occasional variant. One of the leading hypotheses of the preferential transmission of chromosomes with strong centromeres against chromosomes with weak centromeres [91, 92] appears inappropriate because the molecular basis for centromere identity is elusive. These parts of the chromosomes are not supported by specific DNA, and are presumably epigenetically regulated [93, 94]. It is still unknown why and how such a rigid structure as a centromere might have originated through obscure epigenetic mechanisms and survive under natural selection in evolution.

An even more complicated scenario is possible for cases with numerous translocations, especially when distinct translocations emerge in different populations, or when the translocations appeared to be partly homological. The homology by a single branch was named ‘monobrachial’ [95]. There are numerous cases for such translocations in Mus [96], *Rhogeessa*

tumida [97], *Sorex araneus* [98], *Rattus sordidius* [99], rock wallabies [100] and others. In bats, monobrachial translocations were possibly mainstream for speciation [101]. Why did emergences of partial homology in chromosome fusions lead to divergence? The meiosis appeared to be a main barrier for spreading the re-arranged chromosomal sets. In hybrids, during prophase I, the homological chromosomes or their parts synapse in the case of monobrachial homology, and different tetra-, pentavalents or even more complicated figures and chains are puzzled. The proper segregation for the complexes is rather difficult and often leads to abnormal chromosomal sets in metaphase II. The meiotic drive as a biased transmission of genetic variants might be a result of meiotic events during gametogenesis. In female meiosis, homologous chromosomes might be differentially transmitted to the egg or polar bodies. In males, a specific genetic element might disturb the function of sperm that reduces fertility [102-104].

10. Meiotic Troubleshooting in Hybrids with Partial Chromosome Homology

Chromosomal rearrangements, including Robertsonian translocations, can lead to the formation of new balanced karyotypes, nevertheless changing the architectonics of the nucleus. A concept of chromosome territories proposes a non-random distribution of chromosomes in nuclei; a nuclear architecture constitutes the basis for gene expression regulation [105]. The main output is that a transformation of chromatin structures may alter the genetic system of the species due to the modulation accessibility of transcription factors to DNA binding sites, thus regulating gene expression. Robertsonian fusions restructure the organization of the nuclei, especially in the case when chromosome territories of fused chromosomes are located far from each other. The differences in locations may promote the fusions and encourage the meiotic drive in favour of changed chromosomes [106, 107] or against them [108].

In diverse model crossings, which were made for *Mus domesticus*, distinct meiotic disturbances were revealed. For example, different multivalents formed associations with a sex bivalent in prophase I [109, 110]. To evaluate the possible input of the translocations to species diversification, we studied several cases of monobrachial homology in *E. tancrei*, a species with numerous chromosomal forms. The homology of chromosomes was verified by chromosome painting, because G-banding in some cases was not precise enough. The scheme of our experimental hybridization is demonstrated in Figure 2.

We crossed a form with 2n = 50, two pairs of Rbs: 2Rb(2.18) and 2Rb(5.9), which was nicknamed 'Voidara' according to the closest settlement in the Surkhob River Valley (Figure 2b), with two different forms: another form with 2n = 50, distinct two pairs of Rbs: 2Rb(4.12) and 2Rb(9.13), named 'Khodza Obi-Garm' (Figure 2a), and 2n = 48, three pairs of metacentrics: 2Rb(2.11), 2Rb(5.9), and 2Rb(3.18) (Figure 2c). F1 hybrids of the first crossing had 2n=50 and four distinct Rbs (1Rb(2.18), 1Rb(4.12), 1Rb(5.9), and 1Rb(9.13), two of them with monobrachial homology (Figure 2d). In meiotic prophase I, two trivalents and a tetravalent were revealed (Figure 2f). The second crossing resulted in F1 hybrids with 2n=49 and five Rb metacentrics (2Rb(5.9), 1Rb(2.11), 1Rb(2.18) and 1Rb(3.18)), where three of them obtained partial or monobrachial homology (Figure 2e); therefore, a chain (pentavalent) was detected in meiotic prophase I (Figure 2g). These chromosomal forms do not interbreed in nature because they inhabit geographically separated areas in the Pamir-Alay Mountains. In the laboratory, no behavioural differences or preferences for the forms were revealed. Hybrids of the first generation

had lower fertility but did not exhibit any health problems, and their longevity was the same as parental ones (up to seven years). Hybrid fertility increased starting in the third generation after the deep inbreeding depression in the first generation [111, 112].

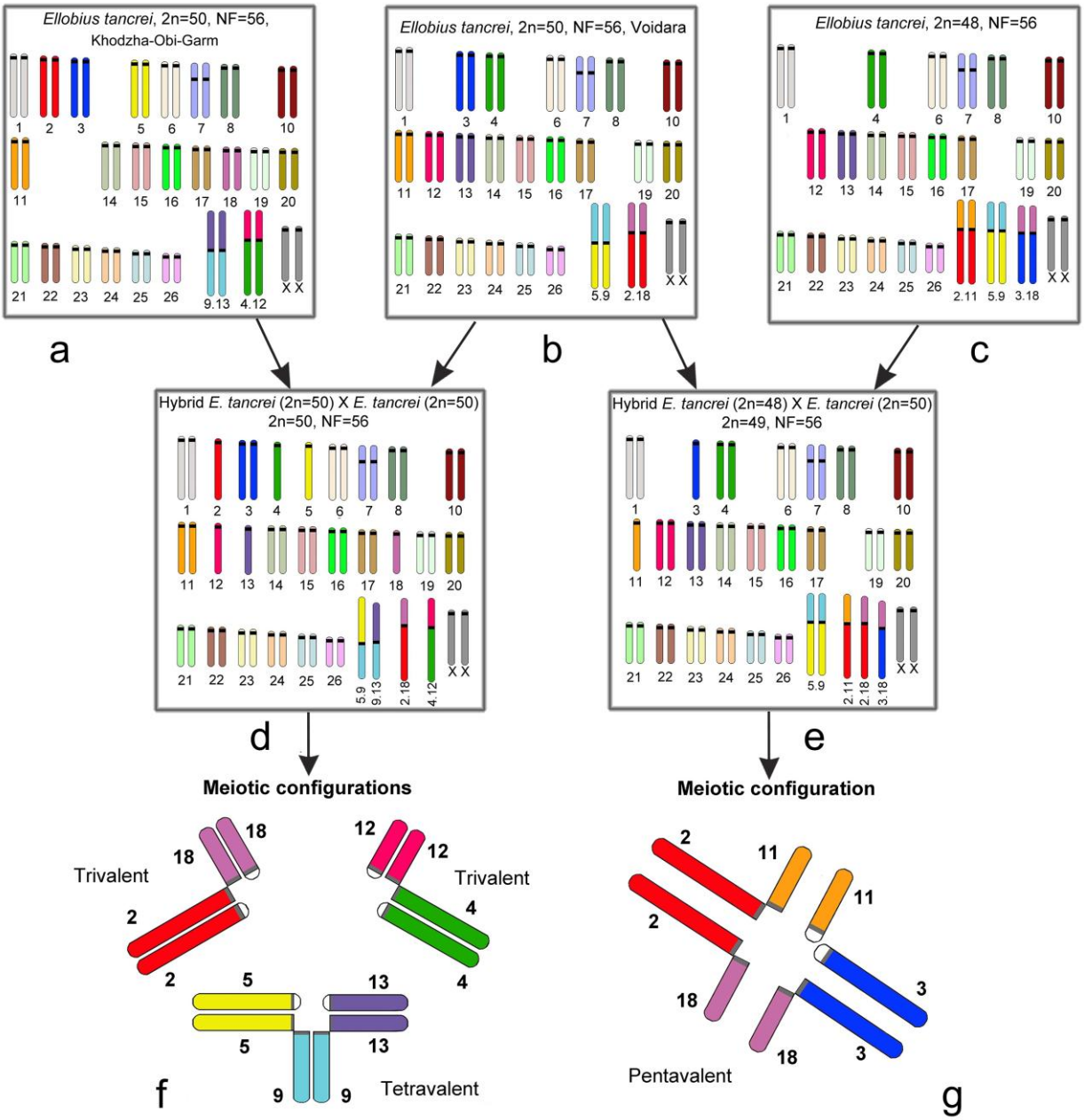

Figure 2 Scheme of experimental hybridization in E. tancrei. **a**. form 'Khodza Obi-Garm', 2n = 50, 2Rb(4.12) and 2Rb(9.13); **b**. form 'Voidara', 2n = 50, 2Rb(2.18), and 2Rb(5.9); **c**. form 2n = 48, 2Rb(2.11), 2Rb(5.9), and 2Rb(3.18); **d**. F1 hybrid with 2n=50, 1Rb(2.18), 1Rb(4.12), 1Rb(5.9), and 1Rb(9.13); **e**. F1 hybrid with 2n=49, 2Rb(5.9), 1Rb(2.11), 1Rb(2.18) and 1Rb(3.18); **f**. the meiotic prophase I of F1 hybrid (2d), with two trivalents and a tetravalent present; **g**. the meiotic prophase I of the F1 hybrid (2e), and the pentavalent consists of 3 Rb metacentrics with monobrachial homology and 2 acrocentrics.

A particularly interesting output was a meiotic solution for heterozygotes by Rb metacentrics. The most common disturbance was delayed synapses, which resumed later if compared to the homologous crossings. The late synaptic adjustments nevertheless provide a proper segregation of chromosomes and normal sets in the gametes. The question of a lower rate of recombination due to delayed synapses is still open; apparently, it may lead to negative consequences in an evolutionary perspective. The role of monobrachial fusions in speciation presumably depends on karyotype organization, and in some cases, may lead to fast divergence, even in several generations, and especially in the case of the physical isolation of hybrids from the parental forms.

11. Conclusion

Loss of the *Sry* gene and hypothetical alteration of the regulation of its target *SOX9* gene in several *Ellobius* and *Tokudaia* species raises a question of universality in the sex determining gene network in placental mammals. A plausible way is an origin of compensatory mutations to correct the disrupted testis determining pathway. One of the possible candidates is the *Cbx2* gene, which might be involved in male sex determination, providing a proper *SOX9* regulation in *Tokudaia* [65]. Different genes were studied as a potential primary sex factor in *Ellobius* and *Tokudaia*, but to date, we do not know how the early development of gonads occurs. Distinctness in the meiotic behaviour of sex chromosomes in *Ellobius* species with two X chromosomes (*E. talpinus* and *E. tancrei*) may be evaluated as a first sign of emergence of new sex chromosomes when X chromosomes in females are fully homological, but in males, these chromosomes demonstrate as typical for heteromorphic sex chromosome behaviour. In a case of sex chromosome origin, and in inheritance of changed autosomes, meiosis appeared to be an essential process for emergence, altering the inheritance of newly originated sex and autosomes [113].

An enigma of when and how Robertsonian translocations may originate will probably get an answer soon. Now, it became possible to study molecular mechanisms of genome instability, and first data on the emergence of translocations in germ lines appeared (see review in [114]). The changes rise in meiosis due to double-strand breaks, and meiosis is also involved in next evolutionary steps through meiotic drive mechanism.

Mole voles *Ellobius,* as exceptions to the rule, are a fruitful model for further studying the evolution of sex determination and chromosomal speciation.

Author Contributions

Authors contributed equally to this work.

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Competing Interests

The authors have declared that no competing interests exist.

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