The origins of the transformed sex chromosome biology of creeping voles through a cascade of adaptive compensatory responses to a selfish X chromosome

Scott William Roy
San Francisco State University
scottwroy@gmail.com

The creeping vole *Microtus oregoni* exhibits remarkably transformed sex chromosome biology, with complete chromosome drive/drag, X-Y fusions, sex reversed X complements, biased X inactivation and X chromosome degradation. Beginning with a selfish X chromosome, I propose a series of adaptations leading to this system, each compensating for deleterious consequences of the preceding adaptation: (i) YY embryonic inviability favored evolution of a selfish feminizing X chromosome; (ii) the consequent Y chromosome transmission disadvantage favored X-Y fusion ("X^P"); (iii) Xist-based silencing of Y-derived X^P genes favored a second X-Y fusion ("X^M"); (iv) X chromosome dosage-related costs in X^PX^M males favored the evolution of X^M loss during spermatogenesis; (v) X chromosomal dosage-related costs in X^M0 females favored the evolution of X^M drive; and (vi) degradation of the non-recombining X^P favored the evolution of biased X chromosome inactivation. I discuss recurrent rodent sex chromosome transformation, and selfish genes as a constructive force in evolution.

Introduction

It has long been known that rodent sex chromosomes exhibit remarkable diversity [1-4]. In contrast to the relative stasis associated with sex chromosomes throughout most placental mammalian families, which largely retain the ancestral sex chromosomes and gene contents that evolved in the placental mammalian ancestor [5,6], albeit with occasional sex chromosome-autosome fusions, different rodent species have undergone diverse innovations (see [7] for an excellent review). Rodent sex chromosomes have repeatedly evolved novelties ranging from non-Mendelian inheritance, to sex chromosome-autosome fusions, to Y chromosomal loss, to turnover of sex determination mechanisms [1-4,8,9]. While most of these anomalies have long been known, the evolutionary origins of these systems remain obscure. It has been speculated that intragenomic conflict between sex chromosomes could drive some or all of these transitions [10], however little empirical or modeling work has attempted to discern sources of conflict and chains of causality underlying such transitions (though see [9,11-12]). In particular, what is needed is stepwise models for the origins of the various systems, particularly given that in many cases it is challenging to imagine how intermediate systems would have functioned, or at least not been eliminated due to severe fitness deficits.

Novel genetic systems are observed across a wide variety of taxa, and range from genome elimination to novel sex determination mechanisms to haplodiploidy to permanent translocation heterozygotes to complex cycles of chromosome drive and drag to balanced lethal sex chromosomes [7,13-17]. Many of these systems present seemingly clear fitness costs, either in their extant form or in evolutionary intermediates bridging the gap from standard systems. The existence of these novelties thus poses a puzzle. Failing a clear adaptive value for the systems themselves, the origins of these systems is often vaguely chalked up to neutral evolution or even fixation of deleterious mutations, or more frequently ignored altogether. However, fixation of deleterious mutations is expected to be vanishingly rare except under very strict population-genetic regimes (i.e., a per-generation fitness cost on the order of the inverse of the effective population size [18]). This regime is extremely unlikely for the sex ratio distortions expected in the evolution of the many novel genetic systems associated with sex determination. Because the commoner sex experiences an average fitness deficit relative to the rare sex close to four times the skew, the costs associated with even a miniscule sex ratio skew exceeds the fixation threshold [19,20]. Large changes in genetic systems also seem unlikely to be strictly neutral, as they impact on a large range of organismal processes (meiosis, development, chromatin packaging, etc.) An alternative force is the action of selfish alleles, which bias transmission towards themselves while reducing the overall fitness of the organisms that carry them. This imposed reduction in fitness clearly provides a selective environment which would favor the fixation of mutations that reduce this fitness cost. If these fitness deficits are large, this may favor the fixation of compensatory mutations even if these compensatory mutations themselves carry substantial costs; these substantial costs could then select for subsequent adaptations, leading to suites of evolutionary novelties. These thoughts suggest that selfish alleles could be an overlooked force driving evolutionary novelty [21-24].

Microtus oregoni represents one of the longest-standing puzzles of sex chromosome biology [25-28], and has been well studied cytologically for decades, documenting a number of changes in *M. oregoni*'s sex chromosomes [25,29-32]. The odd features of *M. oregoni*'s sex chromosomes have attracted speculation as to the origins of the system [10,12,25]. Recently our team published the genome of *M. oregoni* [33]. Together with the cytogenetic findings we now have a much more developed picture of this strangest of mammalian sex chromosome systems, combining two structurally unprecedented sex chromosomes with deeply non-Mendelian inheritance of both chromosomes.

M. oregoni exhibits transformed sex chromosomes. The major features of the system are diagrammed in Figure 1b, with a standard X/Y system diagrammed in Figure 1a for comparison. In place of the standard X and Y chromosomes, *M. oregoni* exhibits two chromosomes that each contain a full X chromosomal gene complement in addition to a complete or near complete set of ancestral Y genes, many of which are present in multiple copies (as well as a small number of ancestrally autosomal genes, which I do not discuss here). These two chromosomes are uniparentally inherited (see below), thus we term them X^P and X^M for the paternally- and maternally-inherited X, respectively. Males are karyotypically X^PX^M and females have a single unpaired X^M. X^P includes a full X chromosomal haplotype along with a nearly-full set of ancestral Y-linked genes. The

association of X^P with maleness suggest that it alone encodes a masculinizing copy of the ancestral mammalian male-determining factor SRY ("s" in Figure 1). X^M also includes a full X chromosomal haplotype along with an apparently full set of ancestral Y-linked genes, though its presence in both sexes suggests that its SRY gene copies are not sufficient for masculinization. Male-specific paternal inheritance of X^P, coupled to universal maternal inheritance of X^M produces X^M0 females and X^PX^M males. (Consistent with the role of the non-coding RNA Xist in ensuring expression of one X chromosome per cell, Xist is expressed in males but not females; remarkably, it appears that the X^P is consistently repressed in males, likely due to disabling promoter mutations in the X^P Xist allele [33]).

M. oregoni also exhibits transformed sex chromosomal inheritance. Our understanding of these processes follows the observations and model of Ohno et al. [25], which centers around X^M non-disjunction events during pre-meiotic diploid stages of gametogenesis occurring in both sexes. In males, a mitotic non-disjunction event produces a viable X^P0 diploid cell and an inviable $X^MX^MX^P$ diploid cell; the former divides and undergo meiosis to yield sperm bearing either X^P or bearing neither chromosome (i.e., either X^P or 0 sperm). In females, a mitotic non-disjunction event produces a viable X^MX^M diploid cell and an inviable 00 diploid cell; the former divides and undergo meiosis to yield eggs each bearing X^M. (Note that both non-disjunction events serve to restore the germline to the typical/ancestral X copy number, that is one copy in males as in XY and two in females as in XX).

Three features of this remarkable system are particularly notable. The first is the sheer number of novelties, including multiple sex chromosomal fusions, presence of ancestral Y-linked genes including SRY in females, non-Mendelian inheritance through both male and female germlines and reversed Xist expression [33]. The second is the remarkable rapidity with which the system has apparently been transformed through evolution, given that no other *Microtus* species are known to exhibit any of these novelties. The third is the degree to which these novelties violate paradigmatic "rules" of mammalian sex chromosome biology [34]. *M. oregoni*'s novelties include expectations that ancestral Y-linked genes disrupt oogenesis, that females lack SRY, that Xist expression is female-specific, that presence of a single X chromosome disrupts oogenesis, and so forth.

In order to understand how the evolutionary constraints acting on mammals generally could have been so dramatically and rapidly overcome in *M. oregoni*, I here interrogate potential selective forces that may have led to the *M. oregoni* system, by developing a stepwise model. I propose that evolution of a selfish feminizing X chromosome kicked off a series of compensatory adaptations, with each adaptation carrying deleterious consequences driving the next compensatory adaptation. Given the sheer number of changes necessary and the complications arising for each one, I have opted to first delineate a simplified schema for the stepwise transformation of a standard mammalian sex chromosome system into the *M. oregoni* system, which is then followed by a separate section discussing the potentially complex population dynamics and the evolutionary loss of standard and intermediate sex chromosomes.

A stepwise model for the origins of the M. oregoni system.

In the following, I use X and Y to represent standard ancestral chromosomes (i.e., with essentially ancestral gene complements and without any of the noted *M. oregoni* novelties); X^* to represent a feminizing X chromosome (see below); and X^P and X^M to represent chromosomes with (nearly) full complements of ancestrally X-linked and Y-linked genes, either with (X^P) or without (X^M) a masculinizing function. These designations follow our previous terminology for *M. oregoni* [33]; however, note that here X^P/X^M are used to indicate masculinizing/non-masculinizing X-Y fusions (or chimeras more generally, regardless of other novel features observed on the extant *M. oregoni* X^P/X^M chromosomes.

Step 1: Inviability of YY progeny drives the evolution of a feminizing X chromosome.

One feature of sex chromosomes that distinguishes rodents from most mammals is the recurrent evolution of so-called feminizing X chromosomes, namely X-linked alleles that cause XY zygotes to develop as female [10,35]. Feminizing X chromosomes are thought to arise because of the transmission advantage enjoyed in XY x XY crosses: because YY progeny lack essential X-linked genes and are thus inviable, X chromosomes in XY females enjoy a transmission advantage, being present in 2/3 instead of 1/2 of viable progeny. This fact provides a selective advantage for X chromosomes that can feminize XY embryos (for a more in-depth description of these dynamics and their condition dependence, see [10,36-37]). I propose that the first step to the evolution of the *M. oregoni* system, beginning with a standard X/Y system (Figure 2a) was the origins of just such a feminizing X chromosome (Figure 2b).

Step 2: Reduced fitness of Y chromosomes due to the action of the feminizing X chromosome drives the evolution of X^P, a masculinizing X-Y chromosome fusion.

Three features of feminizing X chromosome systems suggest that, in the presence of a segregating feminizing X chromosome, a Y chromosome that fused to an X chromosome could benefit (for sake of consistency, let us call this fused, masculinizing chromosome X^P). First, feminizing X chromosomes notably enjoy a transmission advantage directly at the expense of Y chromosomes, specifically due to lack of essential X-linked genes on the Y chromosomes in zygotes carrying two Y chromosomes. This obviously does not apply to a Y chromosome fused to an X chromosome, since an embryo containing two Y chromosomal haplotypes of which at least one is fused to an X chromosome would contain the necessary X-linked genes. Notably, X^PY or X^PX^P males would be expected to have a high proportion of sons, which are particularly valuable given the female-skewed sex ratios caused by the segregating feminizing X chromosome. Second, in some feminizing X systems, oogenesis in X*Y females is biased towards the feminizing X. Given that no bias towards transmission of the feminizing X is not observed in X*X females, it is possible that the presence of X-linked genes (or simply an absence of the specific chromosomal biology of the Y) is protective against drive by the feminizing X chromosome, in which case a X-Y fusion might benefit relative to the non-fused Y by more balanced oogenesis (less drive) in X*-bearing females. Thirdly, whereas X*

expression is expected in all cells in standard X*Y females, in X^*X^P females, the X* is expected to be silenced in half of cells, potentially leading to male development, providing an advantage to the X^P relative to the non-fused Y both given the greater value of males under female-skewed sex ratios, and in avoiding the transmission disadvantage suffered by the Y in X^*Y females.

Thus, I propose that an initial fusion of the X and Y chromosomes to produce a masculinizing fused chromosome occurred, driven by some combination of these forces in response to a segregating X* chromosome. This is shown diagrammatically inof parts b and c Figure 2, with a feminizing X (Figure 2b) driving selection for a fused X-Y chromosome, leading to the situation shown in Figure 2c (note that for Figure 2c only one of the possible crosses is shown; others are shown and discussed in Supplemental Table 1).

Step 3: Xist-based silencing of Y-derived genes leads to costs in X^PX males, driving the evolution of X^M, a non-masculinizing X-Y chromosome fusion.

While the above suggests the possibility that a newly-arising X^P-like fusion could enjoy a benefit and rise in frequency in the population, X^P-containing individuals might also be expected to experience two kinds of costs related to expression of ancestral Y-linked genes. First, assuming random XCI, cells in which the X^P chromosome is silenced might have reduced expression of the Y-derived genes, if Xist sometimes spreads to the Y-derived regions (as appears to be the case in *M. oregoni* [33]). Such costs could be compensated by acquisition of Y-derived genes by the 'standard' X, for instance by a second X-Y fusion or by X-X^P translocation, yielding a proto-X^M chromosome (a full X fused to a full complement of Y-linked genes, though without a masculinizing effect). Such a chromosome could experience an advantage relative to standard X chromosomes by improving function in X^P-bearing males. (Alternatively, the proto-X^M chromosome could simply be produced by loss of the masculinizing function of the X^P, which could then segregate as an X and similarly enjoy an advantage over the standard X.) This is shown in parts c and d of Figure 2, in which silencing of Y-linked genes in some cells of X^PX males (Figure 2c) leads to selection for X chromosomes bearing Y-linked genes (but without a masculinizing function; Figure 2d).

Step 4: Costs related to X chromosome dosage in the germline of X^PX^M males drives X^M nondisjunction and loss during spermatogenesis.

X^PX^M males might initially had imperfect spermatogenesis due to the presence of two X chromosomes. A variety of mammals exhibit subfertility associated with costs in spermatogenesis in atypical males with two X chromosomes (e.g., XXY [38-39]), and it seems likely that similar costs could have been encountered in earlyX^PX^M males. That XX males are standard in at least one rodent lineage indicates that these challenges are not insurmountable [40-41], but it does not follow that such costs would be negligible in newly-evolved X^PX^M males. One possible response could have been evolution of a mechanism to exclude one X chromosome during the early mitotic stages of spermatogenesis. Nondisjunction of the X^M could have alleviated such X chromosomal

dosage costs by reducing the premeiotic cell line to a single X haplotype. While costs related to dosage could equally have been alleviated by elimination of the X^P chromosome, elimination of the non-X^P chromosome could have been favored by female-skewed sex ratios (since X^P-eliminating individuals would have female-biased progeny). The proposed transition is shown in parts d and e of Figure 2, in which dosage-related costs in X^PX^M males (Figure 2d) drives selection for nondisjunction (Figure 2e).

Step 5: Costs related to X chromosome dosage during oogenesis in X^M0 females drives X^M nondisjunction and drive during oogenesis.

Crucial to the viability of the preceding evolutionary step would be the reproductive fitness of the X^M0 females that are expected to be generated as a consequence of production of sperm without a sex chromosome. While, like X^PX^M males, X^M0 females are known to experience reduced fertility in various mammals [42-43], the requirement for two X chromosomes is clearly not particularly severe in species with feminizing X chromosomes (and thus XY females), which presumably would have also been the case if a feminizing X chromosome was an early step in the evolution of the *M. oregoni* system (as proposed above). X0 females are also standard and fertile in at least two other rodent lineages, indicating that these costs are surmountable [44-45]. However, as with the case for XX males, such costs could possibly have been substantial enough to drive an evolutionary response. The observed nondisjunction of the X chromosome during early mitotic divisions in oogenesis could have been such a solution, leading to the restoration of an XX germline in X0 females (Figure 2f).

Step 6: Ongoing degradation of the X^P chromosome drives the evolution of X^P-biased silencing.

Steps 1-5 account for most observed peculiarities of *M. oregoni* sex chromosome biology, leaving loss of Xist expression from the X^M chromosome (i.e., X^P-biased silencing) and loss of some Y-linked genes from the X^P. Both features could have evolved as a response to ongoing degradation of the X^P chromosome. Given the X^P chromosome's inheritance pattern, it is expected to lack recombination, and so is expected to undergo gene loss and general chromosome degradation [46-48]. If recombination between the X^P and X^M never occurred, or if it stopped before the evolution of the X^M nondisjunction in spermatogenesis, the X^P would presumably have begun to degrade, while the X^M would have continued to recombine. Consistent with the X^P ceasing recombination first, more pronounced patterns of degradation are seen on the X^P than on the X^M [33]. Degradation of the X-linked portions of the Y chromosome could have led to selection for silencing of X^P rather than X^M in X^PX^M males (Figure 2f), which could have driven the evolution of the observed putative expression-reducing mutations in the X^M Xist gene promoter (Couger et al. 2021), leading to the observed *M. oregoni* scenario (Figure 2g). However, it is also worth noting that silencing of the X^P has likely also facilitated X^P degradation. Such degradation-promoting forces could also explain the loss of ancestral Y-linked genes from the X^P – such losses would be expected to be well compensated by X^M-linked copies (the exception being genes expressed in the stages of spermatogenesis after spermatogenesis-specific X^M nondisjunction and loss).

Population dynamics of chromosome turnover

The model I have presented is an attempt at a coherent hypothesis for the major evolutionary forces driving the origins of the system. One important omission, which future technical studies should treat, are the specific population dynamics for loss of ancestral and intermediate sex chromosomal forms. In particular, the above discussion leaves reason to think that novel chromosomal forms will experience both advantages and costs relative to the ancestral form from which they arise. That the advantages outweigh the costs (at least when the novel chromosomal form is rare) is a necessary condition for the above model, however the dynamics could be more complex than simple unconditional advantage/disadvantage. If selection on the totality of these effects is frequency dependent, population equilibria retaining multiple chromosomal forms are predicted (e.g., with the ancestral form persisting at some low frequency). Such dynamics are not considered here, but should be considered in future work.

For instance, in the simplest case the X^P could simply enjoy an advantage over the ancestral Y chromosome, thus driving the Y from the population. However, if the Y also enjoys advantages over X^P (e.g., more efficient spermatogenesis in XY males), the Y may be maintained in the population at some frequency (particularly because the ancestral Y's transmission disadvantage only arises in X*Y x XY crosses, which will be rare when the ancestral Y is rare). Similarly, if the X* enjoys no advantage in X^*X^P individuals (e.g., if they are remasculinized or the X* enjoys no transmission advantage), the X* may be driven from the population along with the Y; however so long as the Y persists the X* may enjoy an advantage and persist, leading to a complex equilibrium of X, Y, X*, and X^P chromosomes. Similarly, while in the simplest case the X^M could drive the X to extinction, if the presence of Y-linked genes in females imposes a non-zero cost, the X^M and X could also reach an equilibrium.

Further modeling is clearly desirable. However, it is of note that in several cases it may be expected that further compensatory changes may be expected to reduce the costs experienced by the novel chromosomal forms: selection in XX males, X0 females and ancestral Y-gene-containing females is expected to drive changes that ameliorate these incompatibilities, decreasing the costs accruing to novel chromosomal forms and thus helping them drive the ancestral forms from the population.

Testing the model

While the singular nature of the *M. oregoni* system necessarily limits the direct testing of models of origin, the apparently recurrent evolution of various traits invoked – feminizing X chromosomes, chromosomal fusions, sex chromosome drive – opens up the possibility of testing of at least some of the causalities proposed. More thorough catalogs of the occurrence of various atypical rodent traits would allow for testing of the association between, for instance, feminized XY individuals and masculinized XX individuals (as expected from partial X-Y fusions), as well as associations between fertility of XY females and diverse innovations. Here, the greater accessibility of long

read sequencing will be crucial in characterizing Y chromosomes and other non-recombining regions from a wide variety of rodents. Feedback between observations about rodent sex ratios, karyotyping and genome sequencing across rodents will hopefully lead to the phylogenetic density necessary to reconstruct the order of novelties in atypical rodent lineages, providing the foundations for testing hypotheses about step-wise evolution of odd chromosomal systems.

Selfishness as a constructive force in evolution

Selfish behaviors of genes, from meiotic drive to feminizing X chromosomes, are increasingly appreciated as contributing to the evolution of sex chromosomes in rodents (7, 11, 49-51). Diverse potential outcomes of selfish sex chromosomes have been noted [10], including impacts on the sex chromosomes themselves [51-53], speciation [49,54-56], population size [57], mate choice [58-59], and turnover of sex determining mechanisms. The possibility that selfish sex chromosomes could lead to more profound changes in chromosomal transmission has been less considered (though see [9-10,62]. That segregating selfish alleles drive the evolution of suppressors is widely appreciated [54,63], however another possibility is that the presence of selfish alleles opens up a larger range for counter-adaptation far beyond standard suppression (see many examples in [10]).

A particularly poignant example of how countermeasures other than direct suppression may be expected to evolve is when the action of the selfish allele affects the fitness of other individuals in the population. Such is the case for feminizing X chromosomes, where the action of a feminizing X* chromosome in an X*Y female affects the fitness of her XY male mates, both by producing a female-biased offspring sex ratio and by inviability of YY progeny. XY males do not have the option of directly suppressing the action of the selfish alleles (since they are in the X*Y) female, but will still be selected upon to counter the fitness defects experienced. This scenario could lead to counter measures such as non-Mendelian transmission of sex chromosomes during spermatogenesis [9,62], or potentially to the more exotic possibility of X-Y fusion, as proposed here. In general, the possibility that atypical genetic systems could arise due to countermeasures to the action of selfish alleles is deserving of more detailed attention and building of explicit stepwise models [11,22-23,64-66].

Concluding remarks

I have a proposed a stepwise model that offers adaptive selective explanations for each of the many anomalies reported for *M. oregoni*. While this model is as parsimonious an adaptive model as I can imagine, it is nonetheless quite speculative as well as baroque, and the development of alternatives is clearly desirable. In addition, sequencing of genomes from among the various other atypical rodent sex chromosome systems, as well as scrutiny of close relatives to *M. oregoni* in hopes of ordering some of the *M. oregoni* novelties, will be important for understanding the structures and origins of novel genetic systems.

Acknowledgements: I thank Polly Campbell for helpful comments on the manuscript, and Brian Couger, Noelle Anderson and Landen Gozashti for stimulating conversations. SWR was funded by NSF award 2211661.

Data availability statement: Data sharing not applicable to this article as no datasets were generated or analyzed during the current study

1. Marchal, J. A., Acosta, M. J., Bullejos, M., Díaz De La Guardia, R., & Sánchez, A. (2003). Sex chromosomes, sex determination, and sex-linked sequences in Microtidae. *Cytogenetic and genome research*, *101*(3-4), 266-273.
2. Graves, J. A. M. (2002). Sex chromosomes and sex determination in weird mammals. *Cytogenetic and genome research*, *96*(1-4), 161-168.
3. Fredga, K. (1970). Unusual sex chromosome inheritance in mammals. *Philosophical transactions of the Royal Society of London. B, Biological Sciences*, *259*(828), 15-36.
4. Wurster, D. H., Snapper, J. R., & Benirschke, K. (1971). Unusually large sex chromosomes: new methods of measuring and description of karyotypes of six rodents (Myomorpha and Hystricomorpha) and one lagomorph (Ochotonidae). *Cytogenetic and genome research*, *10*(3), 153-176.
5. Lahn, B. T., & Page, D. C. (1999). Four evolutionary strata on the human X chromosome. *Science*, *286*(5441), 964-967.
6. Brashear, W. A., Bredemeyer, K. R., & Murphy, W. J. (2021). Genomic architecture constrained placental mammal X Chromosome evolution. *Genome Research*, *31*(8), 1353-1365.
7. Saunders, P. A., & Veyrunes, F. (2021). Unusual mammalian sex determination systems: a cabinet of curiosities. *Genes*, *12*(11), 1770.
8. Mulugeta, E., Wassenaar, E., Sleddens-Linkels, E., van IJcken, W. F., Heard, E., Grootegoed, J. A., ... & Baarends, W. M. (2016). Genomes of Ellobius species provide insight into the evolutionary dynamics of mammalian sex chromosomes. *Genome Research*, *26*(9), 1202-1210.
9. Saunders, P. A., Perez, J., Ronce, O., & Veyrunes, F. (2022). Multiple sex chromosome drivers in a mammal with three sex chromosomes. *Current Biology*, *32*(9), 2001-2010.
10. Burt, A., & Trivers, R. (2006). *Genes in conflict: the biology of selfish genetic elements*. Harvard University Press.
11. Roy, S. W. (2021). On the form and origins of the bizarre sex chromosomal system of the mandarin vole. *Journal of Heredity*, *112*(4), 328-334.
12. Charlesworth, B., & Dempsey, N. D. (2001). A model of the evolution of the unusual sex chromosome system of Microtus oregoni. *Heredity*, *86*(4), 387-394.
13. Ross, L., Mongue, A. J., Hodson, C. N., & Schwander, T. (2022). Asymmetric inheritance: the diversity and evolution of non-Mendelian reproductive strategies. *Annual Review of Ecology, Evolution, and Systematics*, *53*, 1-23.

14. Kronauer, D. J. (2023). The unusual genetics of invasive ants. *Science*, *380*(6640), 33-34.
15. Van Goor, J., Shakes, D. C., & Haag, E. S. (2021). Fisher vs. the worms: extraordinary sex ratios in nematodes and the mechanisms that produce them. *Cells*, *10*(7), 1793.
16. Grosmaire, M., Launay, C., Siegwald, M., Brugière, T., Estrada-Virrueta, L., Berger, D., ... & Delattre, M. (2019). Males as somatic investment in a parthenogenetic nematode. *Science*, *363*(6432), 1210-1213.
17. Wielstra, B. (2020). Balanced lethal systems. *Current Biology*, *30*(13), R742-R743.
18. Kimura, M. (1964). Diffusion models in population genetics. *Journal of Applied Probability*, *1*(2), 177-232.
19. Fisher, R.A. (1930). *The Genetical Theory of Natural Selection.* Clarendon Press.
20. West, S. (2009). *Sex allocation*. Princeton University Press.
21. Werren, J. H. (2011). Selfish genetic elements, genetic conflict, and evolutionary innovation. *Proceedings of the National Academy of Sciences*, *108*(supplement_2), 10863-10870.
22. Roy, S. W. (2021). CRISPR-Cas Toxin–Antitoxin Systems: Selfishness as a Constructive Evolutionary Force. *Trends in Microbiology*, *29*(10), 869-870.
23. Normark, B. B., & Ross, L. (2014). Genetic conflict, kin and the origins of novel genetic systems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1642), 20130364.
24. Hurst, G. D., & Werren, J. H. (2001). The role of selfish genetic elements in eukaryotic evolution. *Nature Reviews Genetics*, *2*(8), 597-606.
25. Ohno, S., Beçak, W., & Beçak, M. L. (1963). X-autosome ratio and the behavior pattern of individual X-chromosomes in placental mammals. *Chromosoma*, *15*, 14-30.
26. Pinkel, D., Gledhill, B. L., Lake, S., Stephenson, D., & Van Dilla, M. A. (1982). Sex preselection in mammals? Separation of sperm bearing Y and" O" chromosomes in the vole Microtus oregoni. *Science*, *218*(4575), 904-906.
27. Fredga, K. (1988). Aberrant chromosomal sex-determining mechanisms in mammals, with special reference to species with XY females. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, *322*(1208), 83-95.
28. Johnson, L. A., & Clarke, R. N. (1990). Sperm DNA and sex chromosome differences between two geographical populations of the creeping vole, Microtus oregoni. *Molecular reproduction and development*, *27*(2), 159-162.
29. Ohno, S., Jainchill, J., & Stenius, C. (1964). The creeping vole (Microtus oregoni) as a gonosomic mosaic. I. The OY/XY constitution of the male. *Cytogenetic and Genome Research*, *2*(4-5), 232-239.
30. Modi, W. S. (1987). Phylogenetic analyses of chromosomal banding patterns among the Nearctic Arvicolidae (Mammalia: Rodentia). *Systematic Zoology*, *36*(2), 109-136.
31. Modi, W. S. (1987). C-banding analyses and the evolution of heterochromatin among arvicolid rodents. *Journal of Mammalogy*, *68*(3), 704-714.

32. Libbus, B. L., & Johnson, L. A. (1988). The creeping vole, Microtus oregoni: Karyotype and sex-chromosome differences between two geographical populations. *Cytogenetic and Genome Research*, *47*(4), 181-184.
33. Couger, M. B., Roy, S. W., Anderson, N., Gozashti, L., Pirro, S., Millward, L. S., ... & Campbell, P. (2021). Sex chromosome transformation and the origin of a male-specific X chromosome in the creeping vole. *Science*, *372*(6542), 592-600.
34. Hughes, J. F., & Page, D. C. (2015). The biology and evolution of mammalian Y chromosomes. *Annual review of genetics*, *49*, 507-527.
35. Fredga, K., Gropp, A., Winking, H., & Frank, F. (1976). Fertile XX-and XY-type females in the wood lemming Myopus schisticolor. *Nature*, *261*(5557), 225-227.
36. Bull, J. J., & Bulmer, M. G. (1981). The evolution of XY females in mammals. *Heredity*, *47*(3), 347-365.
37. Bull, J. J. (1983). *Evolution of sex determining mechanisms*. The Benjamin/Cummings Publishing Company, Inc.
38. Yoshida, A., Miura, K., Nagao, K., Hara, H., Ishii, N., & Shirai, M. (1997). Sexual function and clinical features of patients with Klinefelter's syndrome with the chief complaint of male infertility. *international journal of andrology*, *20*(2), 80-85.
39. Mroz, K., Hassold, T. J., & Hunt, P. A. (1999). Meiotic aneuploidy in the XXY mouse: evidence that a compromised testicular environment increases the incidence of meiotic errors. *Human Reproduction*, *14*(5), 1151-1156.
40. Matveevsky, S., Bakloushinskaya, I., & Kolomiets, O. (2016). Unique sex chromosome systems in Ellobius: How do male XX chromosomes recombine and undergo pachytene chromatin inactivation?. *Scientific Reports*, *6*(1), 29949.
41. Matveevsky, S., Kolomiets, O., Bogdanov, A., Hakhverdyan, M., & Bakloushinskaya, I. (2017). Chromosomal evolution in mole voles Ellobius (Cricetidae, Rodentia): Bizarre sex chromosomes, variable autosomes and meiosis. *Genes*, *8*(11), 306.
42. Abir, R., Fisch, B., Nahum, R., Orvieto, R., Nitke, S., & Ben Rafael, Z. (2001). Turner's syndrome and fertility: current status and possible putative prospects. *Human reproduction update*, *7*(6), 603-610.
43. Deckers, J. F. M., Van der Kroon, P. H. W., & Douglas, L. T. (1981). Some characteristics of the XO mouse (Mus musculus L.) II. Reproduction: fertility and gametic segregation. *Genetica*, *57*, 3-11.
44. Nagai, Y., & Ohno, S. (1977). Testis-determining HY antigen in XO males of the mole-vole (Ellobius lutescens). *Cell*, *10*(4), 729-732.
45. Sutou, S., Mitsui, Y., & Tsuchiya, K. (2001). Sex determination without the Y chromosome in two Japanese rodents Tokudaia osimensis osimensis and Tokudaia osimensis spp. *Mammalian genome*, *12*(1), 17-21.
46. Charlesworth, B., Morgan, M. T., & Charlesworth, D. (1993). The effect of deleterious mutations on neutral molecular variation. *Genetics*, *134*(4), 1289-1303.
47. Bachtrog, D., Hom, E., Wong, K. M., Maside, X., & de Jong, P. (2008). Genomic degradation of a young Y chromosome in Drosophila miranda. *Genome biology*, *9*, 1-10.

48. Rice, W. R. (1987). The accumulation of sexually antagonistic genes as a selective agent promoting the evolution of reduced recombination between primitive sex chromosomes. *Evolution*, *41*(4), 911-914.
49. Roy, S. W. (2022). Haldane's duel: intragenomic conflict, selfish Y chromosomes and speciation. *Trends in Genetics*, *38*(1), 8-11.
50. Rathje, C. C., Johnson, E. E. P., Drage, D., Patinioti, C., Silvestri, G., Affara, N. A., ... & Ellis, P. J. I. (2019). Differential sperm motility mediates the sex ratio drive shaping mouse sex chromosome evolution. *Current Biology*, *29*(21), 3692-3698.
51. Kruger, A. N., Brogley, M. A., Huizinga, J. L., Kidd, J. M., de Rooij, D. G., Hu, Y. C., & Mueller, J. L. (2019). A neofunctionalized X-linked ampliconic gene family is essential for male fertility and equal sex ratio in mice. *Current Biology*, *29*(21), 3699-3706.
52. Bachtrog, D. (2020). The Y chromosome as a battleground for intragenomic conflict. *Trends in Genetics*, *36*(7), 510-522.
53. Ellison, C., & Bachtrog, D. (2019). Recurrent gene co-amplification on Drosophila X and Y chromosomes. *PLoS genetics*, *15*(7), e1008251.
54. Frank, S. A. (1991). Divergence of meiotic drive-suppression systems as an explanation for sex-biased hybrid sterility and inviability. *Evolution*, *45*(2), 262-267.
55. Patten, M. M. (2018). Selfish X chromosomes and speciation. *Molecular ecology*, *27*(19), 3772-3782.
56. O'Neill, M. J., & O'Neill, R. J. (2018). Sex chromosome repeats tip the balance towards speciation. *Molecular Ecology*, *27*(19), 3783-3798.
57. Mackintosh, C., Pomiankowski, A., & Scott, M. F. (2021). X-linked meiotic drive can boost population size and persistence. *Genetics*, *217*(1), iyaa018.
58. Reinhold, K., Engqvist, L., Misof, B., & Kurtz, J. (1999). Meiotic drive and evolution of female choice. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *266*(1426), 1341-1345.
59. Finnegan, S. R., Nitsche, L., Mondani, M., Camus, M. F., Fowler, K., & Pomiankowski, A. (2020). Does meiotic drive alter male mate preference?. *Behavioral Ecology*, *31*(1), 194-201.
60. Úbeda, F., Patten, M. M., & Wild, G. (2015). On the origin of sex chromosomes from meiotic drive. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1798), 20141932.
61. Yoshida, K., & Kitano, J. (2012). The contribution of female meiotic drive to the evolution of neo-sex chromosomes. *Evolution*, *66*(10), 3198-3208.
62. Romanenko, S. A., Smorkatcheva, A. V., Kovalskaya, Y. M., Prokopov, D. Y., Lemskaya, N. A., Gladkikh, O. L., ... & Graphodatsky, A. S. (2020). Complex structure of Lasiopodomys mandarinus vinogradovi sex chromosomes, sex determination, and intraspecific autosomal polymorphism. *Genes*, *11*(4), 374.
63. Tao, Y., Masly, J. P., Araripe, L., Ke, Y., & Hartl, D. L. (2007). A sex-ratio meiotic drive system in Drosophila simulans. I: an autosomal suppressor. *PLoS biology*, *5*(11), e292.
64. Normark, B. B. (2003). The evolution of alternative genetic systems in insects. *Annual review of entomology*, *48*(1), 397-423.

65. Vea, I. M., de la Filia, A. G., Jaron, K. S., Mongue, A. J., Ruiz-Ruano, F. J., Barlow, S. E., ... & Ross, L. (2021). The B chromosome of Pseudococcus viburni: a selfish chromosome that exploits whole-genome meiotic drive. *bioRxiv*, 2021-08.
66. Ross, L., Pen, I., & Shuker, D. M. (2010). Genomic conflict in scale insects: the causes and consequences of bizarre genetic systems. *Biological Reviews*, *85*(4), 807-828.

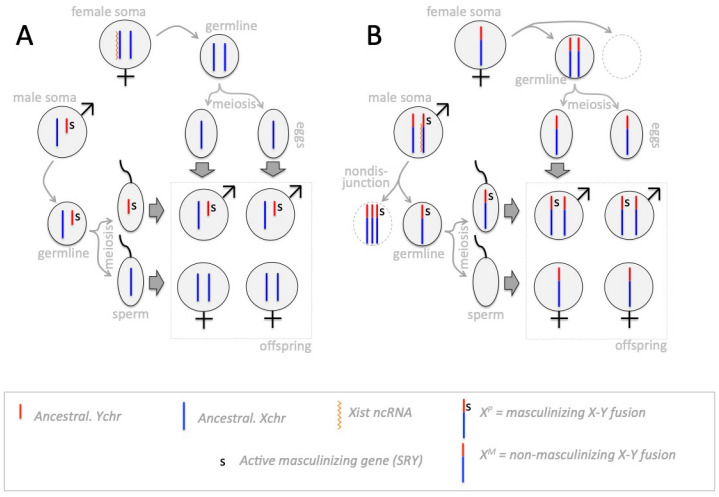

Figure 1. Schematic of the atypical *M. oregoni* sex chromosomal system. Crosses between a typical female (right of the top left corner) and a typical male (below top left corner) are shown, including germline and gametes. Meiosis schematics depict all possible gamete types, not the products of a single meiosis. A. Standard mammalian XX/XY systems. B. X^PX^M/X^M0 system in *M. oregoni*.

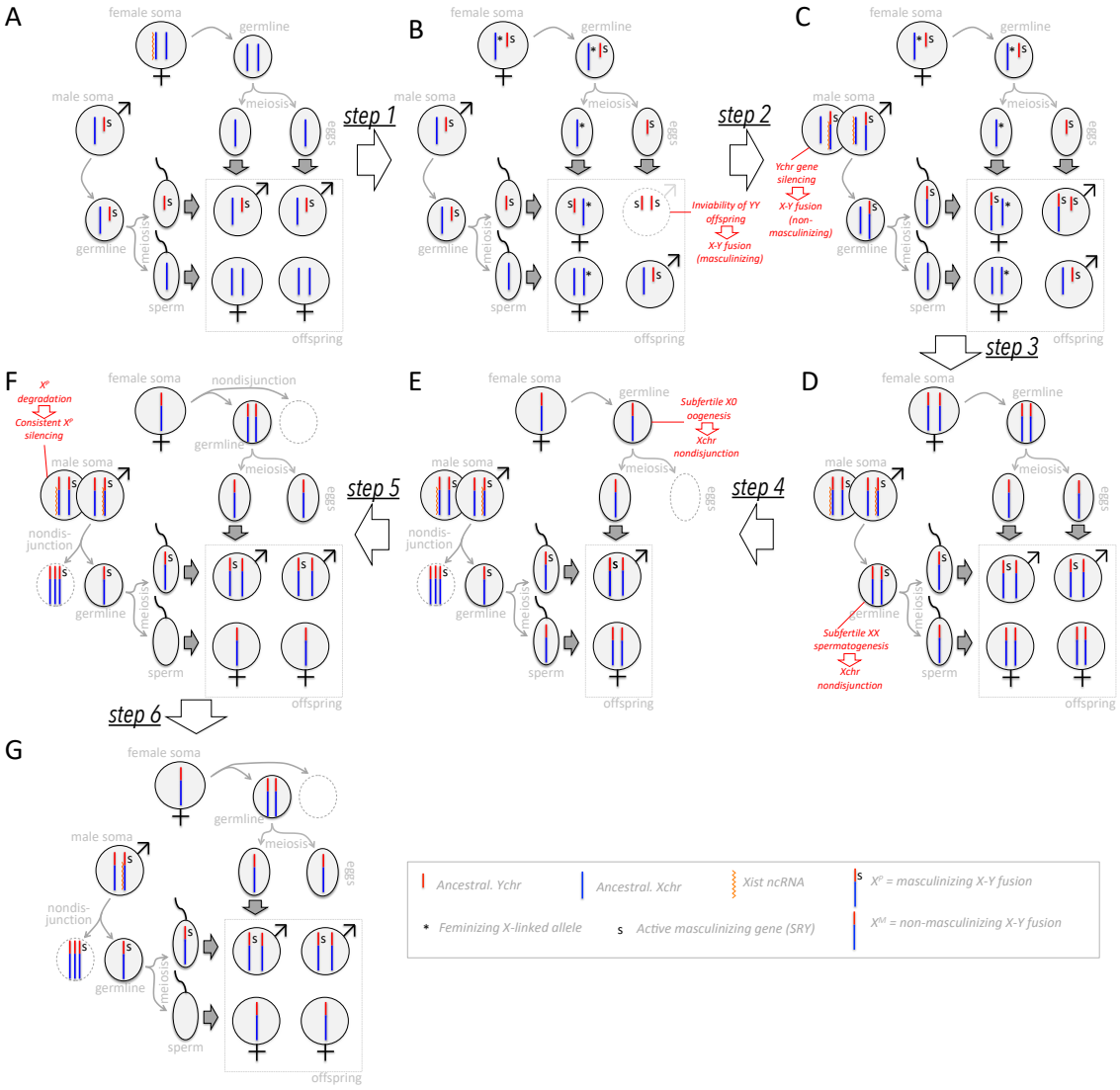

Figure 2. Potential stepwise model for the origins of the *M. oregoni* system, diagrammed as describe in Figure 1. Starting from the ancestral sex chromosome cycle (A), in Step 1 a feminizing X chromosome (X*) arises, gaining an advantage due to inviability of YY offspring (B). In Step 2, inviability off YY offspring drives an X-Y fusion (X^P chromosome) (C; only one possible cross is shown, with others given in Supplemental Table 1). In Step 3, stochastic silencing of ancestral Y-linked genes in X^PX males leads to a second X-Y fusion to yield a non-masculinizing X^M chromosome (D). In Step 4 subfertility of X^MX^P males drives selection for a male germline mitotic X^M nondisjunction event; death of $X^PX^MX^M$ cells leads to X^P0 diploid germline cells and X^P- or 0-bearing sperm (E). In Step 5, subfertility of X^M0 females produced by 0-bearing sperm drives selection for a female germline mitotic X^M nondisjunction of 00 cells leads to X^MX^M diploid germline cells and universally X^M-bearing eggs (F). In Step 6, degradation of the non-recombining X^P chromosome leads or selection to only express X^M alleles in males, and thus to disabling of the Xist promoter on the X^M chromosome. G. The extant *M. oregoni* chromosomal cycle.

Supplemental Table 1. All possible crosses during the second stage of the proposed evolutionary pathway leading to the M. oregoni system (see Figure 2c). The 16 possible crosses between four possible genotypes of fathers (red) and four possible genotypes of mothers (blue) are shown, as are the offspring genotypes, viability, and sex, with chromosomes in offspring colored to indicate parent of origin.