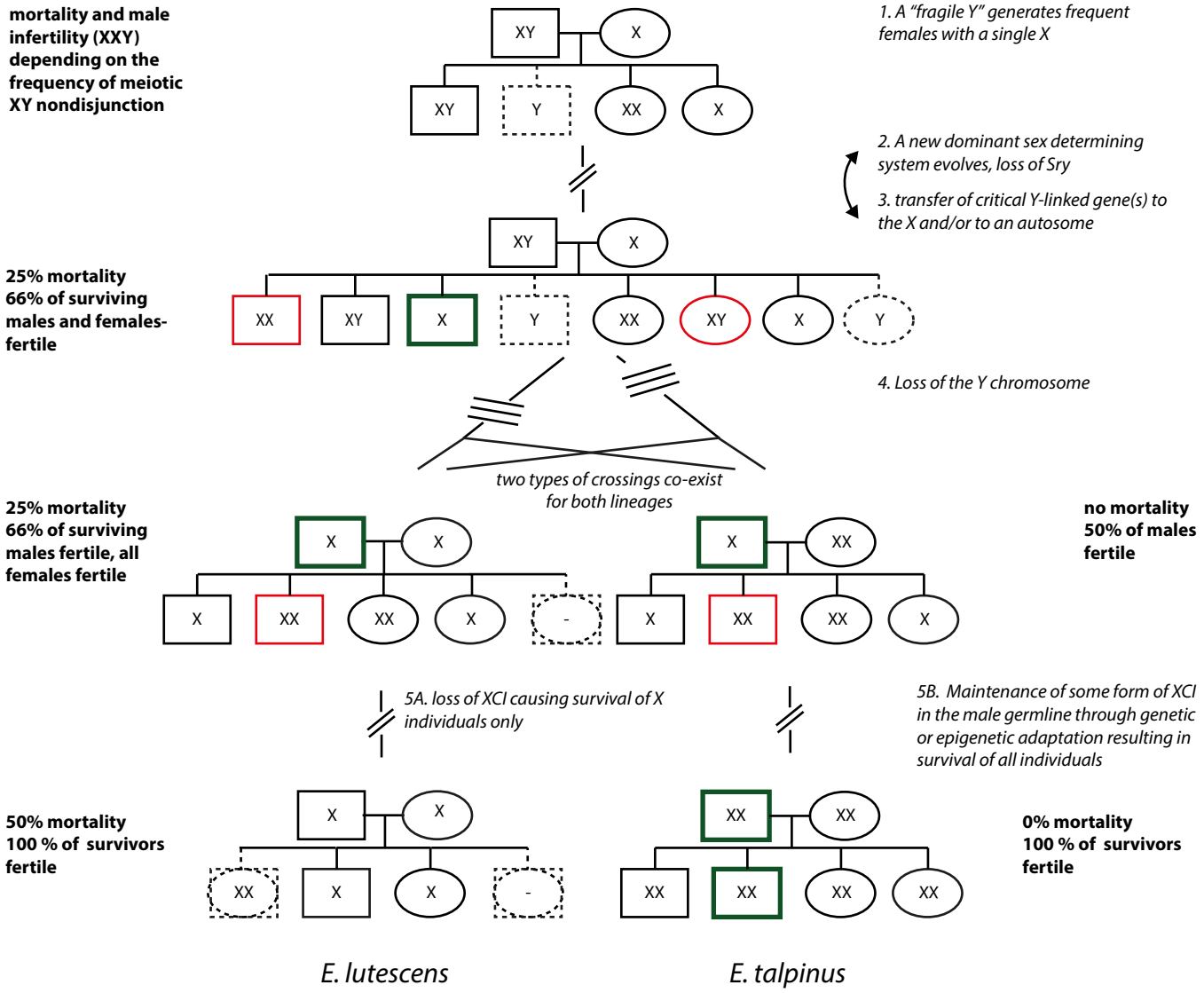

Supplemental Figure S9: Possible course of events during evolution of the X (*lutescens)* and XX (*talpinus*) *Ellobius* species

In the XX/XY ancestor with sex determination initiated by *Sry* expression, meiotic XY nondisjunction is a frequent occurence due to the presence of a very short PAR, generating a "fragile Y"[1]. This results in frequent generation of XXY males (not shown, mostly infertile) and fertile females with a single X (point 1). Then a new dominant sex determining system arises, whereby surviving males (squares) and females (ovals) can be X XX or XY (point 2). In this situation, XX males and XY females will be infertile. Transfer of essential or spermatogenesis genes from the Y Chromosome to the X Chromosome or to an autosome (point 3, but this may also occur already prior to evolvement of a new sex-determining system, indicated by the arrow) may give rise to fertile X males that have lost the Y as a result from the frequent non-disjunction event in the male germ line (points 1 and 4). Loss of the Y will then be beneficial, since it is no longer required for maleness or male fertility, and interferes with female fertility. Crossings of X males with XX or X females indicated in the middle two pedigrees will allow all females (XX and X) to be fertile, but lead to mortality of embryos that have no X Chromosome at all. Since more X than XX females will be born, X individuals may gradually outnumber XX individuals. In the *E. lutescens* lineage (left lower pedigree), loss of XCI will increase embryo mortality, but all surviving offspring will have a 17,X karyotype and be fertile (point 5A). The trade-off between infertility and embryo mortality may have depended on several parameters such as population density, mating behavior, and whether or not litter size was mainly determined by the availability of food. The latter may be an important parameter in the case of the mole vole that requires a relatively high energy input to sustain life underground. *E. talpinus* lost the Y chromosome independent from *E. lutescens*, but a transient X/XX system may also have existed, that was most likely followed by an evolutionary adaptation of X chromosome regulation (point 5B). Normally, in embryonic primordial germ cells of mammals, the inactive X is reactivated in XX individuals. Perhaps *E. talpinus* has developed a mechanism that allows maintenance of one inactive X, specifically in the male, but not in the female germ line (oocyte development proceeds better in the presence of two active X chromosomes). This would then allow survival of male spermatogonial XX stem cells. Thus, XCI may have been lost in *E. lutescens*, and sex-specifically adapted in *E. talpinus,* to generate fertile Y-less males. It should be noted that steps 2-4 most likely occurred (at least) twice; once generating *E. talpinus*, and a few million years later leading to evolvement of *E. lutescens*. Lethality is indicated by dashed lines, infertility by red lines, and green lines indicate fertility arising due to evolutionary changes.

[1]Blackmon H, Demuth JP. 2015. The fragile Y hypothesis: Y chromosome aneuploidy as a selective pressure in sex chromosome and meiotic mechanism evolution. Bioessays 37: 942-950.