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SEX REVERSAL: A FOUNTAIN OF YOUTH FOR SEX CHROMOSOMES?

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Nonrecombining Y chromosomes are expected to degenerate through the progressive accumulation of deleterious mutations. In lower vertebrates, however, most species display homomorphic sex chromosomes. To address this, paradox I propose a role for sex reversal, which occasionally occurs in ectotherms due to the general dependence of physiological processes on temperature. Because sex-specific recombination patterns depend on phenotypic, rather than genotypic sex, homomorphic X and Y chromosomes are expected to recombine in sex-reversed females. These rare events should generate bursts of new Y haplotypes, which will be quickly sorted out by natural or sexual selection. By counteracting Muller's ratchet, this regular purge should prevent the evolutionary decay of Y chromosomes. I review empirical data supporting this suggestion, and propose further investigations for testing it.

KEY WORDS: **Ectothermy, Muller's ratchet, recombination, sex-antagonistic genes, sex determination.**

The Announced Death of Y Chromosomes

Born to be destroyed, such is the tragic destiny of Y chromosomes—or at least such a claim is often made in the literature (e.g., Steinemann and Steinemann 2005). However, to paraphrase Mark Twain, rumors of these deaths might have been greatly exaggerated.

According to a widely accepted view, the recruitment of a new sex chromosome into a preexisting system starts with the appearance on an autosome, by mutation or translocation, of a gene affecting the sex-determining cascade, such that heterozygotes develop into one sex whereas homozygotes develop into the other one (Ohno 1967; Rice 1996; Charlesworth and Charlesworth 2000). Mutations with sexually antagonistic effects will then be favored in the vicinity of this gene, benefitting from linkage disequilibrium. To preserve epistatic interactions, recombination between such mutations and the sex-determining locus will then be suppressed in the heterogametic sex (Bull 1983; Rice 1987). Note that, when evolving from hermaphroditism, a nascent sex-determination system requires two separate mutations (one suppressing male fertility, the other female fertility), which obviously provide additional incentives to prevent recombination (Charlesworth et al. 2005).

This, however, will have strong side effects on all genes that happen to be trapped in the nonrecombining segment. Enhanced genetic drift, combined with background selection and selective sweeps, will accumulate deleterious mutations. Muller's ratchet will condemn these genes to a progressive decay, except for the few that are essential for male development. The process may actually not stop here, because linkage disequilibrium may favor accumulation of further sex-antagonistic mutations at the border of this nonrecombining segment, driving its progressive expansion along the chromosome, followed by its inevitable decay. The whole Y chromosome may thus progressively degenerate, except for small pseudo-autosomal regions often required for proper meiotic segregation (Charlesworth and Charlesworth 2000).

This model accounts for many features of the old sex chromosomes presently found in birds and mammals, including the several evolutionary strata corresponding to the progressive expansion of the nonrecombining segment (Lahn and Page 1999; Lawson Handley et al. 2004). Born some 200 million years (My) ago, these chromosomes nowadays display extreme

heteromorphy. But this situation is in a striking contrast with that found in lower vertebrates (fish, amphibians and reptiles), in which heteromorphic sex chromosomes are the exception rather than the norm (Schartl 2004). In amphibians, for instance, all species investigated so far display genetic sex determination, but more than 96% have homomorphic sex chromosomes (Schmid et al. 1991; Eggert 2004). Similar numbers are to be found in fish (e.g., Devlin and Nagahama 2002).

The reason generally invoked for this lack of differentiation assumes a high turnover of sex chromosomes. New master sex-determining genes are supposed to regularly appear on autosomes, replacing previously established sex chromosomes before they have time to accumulate deleterious mutations or structural changes (Schartl 2004; Volff et al. 2007). There is indeed evidence that turnovers recently occurred in several fish species (e.g., Kondo et al. 2004; Peichel et al. 2004; Volff et al. 2007; Baroiller et al. 2009). In amphibians, different populations from the same species have been shown to display different heterogametic systems (Miura 2007), and additional indirect evidence comes from the phylogenetic analysis of Hillis and Green (1990), who identified seven heterogametic transitions (i.e., transitions from male to female heterogamety or vice versa) during amphibian evolutionary history.

The point might be made, however, that seven transitions during the old evolutionary history of this species-rich group still leaves plenty of time for Y or W to decay, even assuming that some turnovers did not affect heterogamety. Turnover certainly plays a role in the prevalence of homomorphic sex chromosomes, but might not be the whole story. Did more than 96% of amphibian species experience a recent turnover? This seems unlikely, and, as the present article will suggest, it does not need to be so. Contrasting with the high-turnover model, which preserves the main idea that Y chromosomes necessarily decay (until they are replaced), I will propose that "young" (i.e., homomorphic) sex chromosomes might actually harbor old sex-determining genes. This "old-wine-in-a-new-bottle" model relies on two arguments. First, recombination patterns depend on phenotypic sex, rather than on genotypic sex. Second, sex reversal is easily achieved and sometimes spontaneously occurs in lower vertebrates. It follows that X and Y chromosomes are expected to recombine occasionally in sex-reversed XY females. As developed below, such rare events should have far reaching consequences on the evolution of sex chromosomes, preventing the decay and announced death of Y chromosomes.

Recombination Patterns Depend on Phenotypic Sex, Not on Genotypic Sex

There is no doubt that nonrecombination is expected to induce decay of the Y (to an extent that increases with the size of the region of suppressed exchange). But the underlying mechanisms are of crucial importance for the present argument. What prevents X and Y from recombining in males? Because these two chromosomes are so differentiated in mammals, the response seems obvious: of course they cannot recombine, they are too different. However, the common notion that nonrecombination derives from structural changes may mix up causes and consequences. As argued below, structural differences may have actually accumulated because of nonrecombination.

Structural differences are not required to induce sex-differences in recombination. In most species, autosomes, which are shared by both sexes, also display sex-specific patterns of recombination (Burt et al. 1991), a pattern referred to as heterochiasmy, which affects both the rate and localization of crossovers (Lenormand 2003; Lenormand and Dutheil 2004). The overall rate of recombination in human females, for instance, is about twice that of males, but males exhibit significantly more recombination in telomeric regions. The ratio of female-to-male recombination rate varies enormously among animals (from 0.14 to 14; Coimbra et al 2003; Berset-Brändli et al. 2008), not to mention achiasmate species such as found in Diptera (e.g., *Drosophila melanogaster*; Morgan 1914) or in Lepidoptera (e.g., the silkworm *Bombyx mori*; Tanaka 1914), in which one sex does not recombine at all. Which sex recombines more varies among taxa and loci, but heterochiasmy itself is a pervasive feature of sexually reproducing species, including those with environmental sex-determination: females are genetically identical to males in salt-water crocodiles (*Crocodylus porosus*), but display distinct recombination patterns (Isberg et al. 2006).

That sex-specific recombination patterns may depend on phenotypic sex, rather than on sex-specificities of genomic structures, is clearly confirmed by sex-reversal experiments. In the crested newt *Triturus cristatus*, sex-reversed XY females "show the same procentric localization of chiasmata that is characteristic of normal females and that is clearly different from the more distal chiasmata that are found in males. The position of chiasmata therefore depends on actual sex rather than genetic sex" (Wallace et al. 1997). Sex-reversed XY females in the medaka fish *Oryzias latipes* similarly display the characteristic recombination patterns of XX females, quite different from those of XY males (e.g., Kondo et al. 2001). Sex-reversed neomale tilapias display male-specific synaptonemal complexes (Campos-Ramos et al. 2009), which are known to drive sex-specific differences in recombination rates (Tease & Hulten 2004). The same appears to be true of autosomal recombination in higher vertebrates: in sex-reversed XY female mice, Lynn et al. (2005) found that "the rate and pattern of recombination in XY oocytes were virtually identical to those in normal XX females, indicating that sex, not genotype, is the primary determinant of meiotic recombination patterns in mammals."

It is worth noting that this remarkable characteristic of sex-reversed XY females of having female-specific (as opposed to genotypic-specific) recombination patterns is being exploited for mapping male-determining genes in the housefly *Musca domestica*, due to the extremely low recombination rate in XY males (e.g., Inoue et al. 1983). The same dependence on phenotypic sex is actually to be seen on young sex chromosomes. Male medaka fish normally display bright colors due to a sex-antagonistic coloration gene situated on the small nonrecombining segment of their nascent sex chromosomes. In the d-rR strain, the dominant allele *R* (orange-red) is fixed on the Y chromosome whereas the recessive allele *r* (white) is fixed on the X. Mating sex-reversed orange-red females (X^rY^R) with sex-reversed white males (X^rX^r), Yamamoto (1961) found an unexpected proportion of orange-red females (X^RX^r) and white males (X^rY^r) among the progeny, resulting from crossovers in the XY mother. Using additional sex-linked markers, Matsuda et al. (1999) confirmed that sex-reversed XY females display female-specific recombination patterns on the sex chromosomes, and further showed that sex-reversed XX males (as well as YY males produced by mating sex-reversed XY females with normal XY males) display the male-specific pattern of restricted recombination. As Matsuda et al. (1999) conclude, "the recombination restriction of the sex chromosomes in heterogametic males does not result from heterogametic sex chromosomes, but from maleness."

In more differentiated sex chromosomes, structural changes may have accumulated to such a point that recombination is not possible anymore in sex-reversed females. However, there is clear evidence from several detailed studies that structural changes followed the cessation of recombination, rather than causing it. In human sex chromosomes, the Amelogenin gene, situated at the boundary between evolutionary strata 3 and 4, testifies to a progressive expansion of the nonrecombining segment. Its 5′ end stopped recombining before the split between primates and ungulates whereas its 3′ end stopped after the split between apes and monkeys (Iwase et al. 2003). Inversions were not involved in this process, because none of the species investigated display a truncated Y copy. "Thus, the suppression of X–Y recombination may have proceeded by another, as yet unidentified mechanism" (Marais and Galtier 2003). Inversions do occur on differentiated sex chromosomes, but might often do so after recombination has stopped. From their study of the rearrangements on *Silene latifolia* Y chromosome, Bergero et al. (2008) conclude that the Y chromosome of this species has been derived through multiple rearrangements of the ancestral gene arrangement, but that "none of the rearrangements so far detected was involved in stopping X–Y recombination."

The role of structural changes as initial causes to nonrecombination might thus have been overemphasized. The exact mechanisms underlying sex-differences in recombination might often relate to differences in the physiological processes of male versus female meiosis (Tease and Hulten 2004), as well as in the epigenetic patterns of methylation and imprinting. Imprinted regions, for example, have been shown to display large sex-differences in recombination rates (Smalley 1993; Paldi et al. 1995; Robinson and Lalande 1995; Lercher and Hurst 2003). This point certainly deserves greater empirical scrutiny.

Phenotypic and Genotypic Sex Are Easily Decoupled in Lower Vertebrates

The second piece in the argument is that sex can easily be reversed in lower vertebrates. Many fish produce highly male-biased sex ratios in response to even small increases in temperature, including species with differentiated sex chromosomes (e.g., Sato et al. 2005; review in Ospina-Álvarez and Piferrer 2008). All amphibian species are believed to have genetic sex determination, but sex can be easily reversed by temperature (Dournon et al. 1990; Wallace et al. 1999). In crested newts, for instance, clutches produce XY sex-reversed females when reared at high temperature, and XX sex-reversed males when reared at low temperature (Wallace and Wallace 2000). Sex reversal is likely to be mediated by the temperature dependence of aromatase (or aromatase inhibitors), which transforms testosterone into oestradiol (e.g., D'Cotta et al. 2001). A direct application of oestradiol has a similar feminizing effect in fish, amphibians, and reptiles (e.g., Bull et al. 1988; Pandian and Sheela 1995; Wallace et al. 1999; Ramsey and Crews 2009) including species with heteromorphic sex chromosomes (Freedberg et al. 2006).

The same dependencies may actually underlie the physiological processes of sex determination in endotherms: chickens can be sex-reversed, not only by hormonal treatment (e.g., Vaillant et al. 2001; Yang et al. 2008) but also by altering ambient temperature during the sex-sensitive time window of embryonic development (a feature exploited by poultry breeders; United States patent 5575237, http://www.freepatentsonline.com/5575237.html). Homeothermy normally prevents expression of these environmental effects, with possible exceptions however: temperature has been shown to affect sex ratio in the mound-building megapode *Alectura lathami*, with more males hatching at low incubation temperatures (31°C) and more females at high temperatures (36°C; Göth and Booth 2005).

In lower vertebrates, spontaneous sex reversal definitely occurs in nature. There is mounting evidence for mixed sex-determination systems (where genetic determination is overridden by temperature within the natural range) in lizards (Shine et al. 2002; Quinn et al. 2007; Radder et al. 2008) and fish (Baroiller et al. 2009; review in Ospina-Alvarez and Piferrer 2008), including species with differentiated sex chromosomes. Sex reversal

also occurs spontaneously in species considered to have purely genetic sex determination. Medaka fish normally display a XY sex determination system, but spontaneously occurring XY females have been reported (e.g., Aida 1936). When mated with normal XY males, such females produce male-biased sex ratios of 1:3, including YY males that sire all-male broods. Nagler et al. (2001) report an event of mass feminization in a population of Chinook salmon, possibly linked to a temperature increase induced by a dam upstream of the spawning grounds. Sex-reversed XY females were fully fertile, producing YY males. Similar events are reported from amphibians. Kawamura and Nishioka (1977) report findings of a sex-reversed XY female *Hyla japonica*, as well as three YY males (all albinos) that necessarily stemmed from fertile, sex-reversed XY females. All turned out to be fertile, the XY female producing clutches with male-biased sex ratios (1:3) including YY males, and the YY males siring all-male progenies. Matsuba et al. (2008) recently reported an event of mass feminization in a northern-Finland population of *Rana temporaria*, a species in which spontaneous sex-reversal had already been documented (Crew 1921; Witschi 1929). The sex ratio of breeding adults was strongly female-biased in years 1999–2000, with a progressive return to an even sex ratio thereafter. Population and family studies using a sex-linked genetic marker showed evidence of large-scale sex reversal (see below).

X and Y Are Expected to Recombine in Sex-Reversed Females

From the above argument, X and Y chromosomes are expected to recombine in naturally occurring sex-reversed females. Datasets allowing tests of this expectation are still lacking, but the quantitative information and sibship analyses provided by Matsuba et al. (2008) deliver relevant insights. These authors collected clutches from a northern-Finland population (Kilpisjärvi) displaying sex-reversal and female-biased sex ratios, as well as from a control population displaying normal sex ratios (Helsinki). Juveniles were reared through metamorphosis at ca 20°C until they had completed their gonadal development, then dissected to determine phenotypic sex, and genotyped at a sex-linked locus (*RtSB03*). Table 1A presents data for a clutch from the control Helsinki population, showing an even sex ratio and a clear correlation between genotypes and phenotypic sex: allele *07* was present in all sons, and only in sons. The straightforward interpretation is that the mother was heterozygote *X 04/ X 10b*, and the father *X 04/ Y 07*, with no recombination between the sex-determining locus *Y* and the marker *RtSB03*.

In contrast, the clutches from Kilpisjärvi displayed biased sex ratios, and unexpected sex-genotype associations. In family Kil-7, for instance, allele *09* was found only in sons, but many sons did not possess it (Table 1B). Inferred maternal and paternal *RtSB03* genotypes were *13*/*04* and *11*/*09*, respectively, with allele *09* on the paternal Y. I propose that these data are best interpreted by assuming, first, that the mother was a sex-reversed XY female; second, that X and Y chromosomes recombined in this female (though not in the male). According to this interpretation, the father produced two kinds of gametes (*X 11* and *Y 09,* displayed in the two lines of Table 1c) but the mother produced four kinds (*X 13*, *X 04*, *Y 13*, and *Y 04*, displayed in the four columns of Table 1C), in proportions depending on the female recombination rate between the sex-determining locus and the marker *RtSB03*. Their mating then produced one quarter of XX females with genotypes *13/11* and *04/11*, one quarter of XY males with the same genotypes, one quarter of XY males with genotypes *13/09* and *04/09*, and one quarter of YY males with the same *RtSB03* genotype. This interpretation explains not only the genotypes produced, but also their numbers; in particular the observed sex ratio (12 daughters and 29 sons) nicely fits the 1:3 ratio expected. It also predicts that half of the males with genotypes *13/09* and *04/09* were YY individuals.

Sex-reversal by itself may explain the original female bias in adult sex ratio (through sex-reversal of genetic males) and the ensuing male-biased sex ratio of offspring (through the excess male production by XY females), but XY recombination is required in addition to explain the peculiar patterns of sex-genotype association found in some families (see above) and the overlap of allelic

Table 1. ***RtSB03*** **genotypes and phenotypic sex of** ***R. temporaria*** **offspring from (A) one clutch of a control population with even sex ratios (Helsinki) and (B) one clutch of a Northern-Finland population displaying sex reversal (Kil-7). After Matsuba et al. (2008). (C) The observed patterns of sex-genotype association and sex ratio at Kil-7 seem best explained by assuming that the mother was a sex-reversed XY female (*** ***RtSB03*** **genotype** ***13/04***) **and produced four kinds of gametes through X–Y recombination (columns), whereas the XY father (*** ***RtSB03*** **genotype** ***11/09***) **only produced** ***X 11*** **and** ***Y 09*** **gametes (lines) due to the lack of recombination.**

(A) Helsinki	*n*	*04/04*	*10b/04*	*04/07*	*10b/07*
Female	13	8	5	0	0
Male	10	0	0	5	5

(B) Kil-7	*n*	*04/11*	*13/11*	*04/09*	*13/09*
Female	12	4	8	0	0
Male	29	8	3	6	12

(C) Kil-7	*X 13*	*X 04*	*Y 13*	*Y 04*
X 11	*XX 13/11*	*XX 04/11*	*YX 13/11*	*YX 04/11*
Y 09	*XY 13/09*	*XY 04/09*	*YY 13/09*	*YY 04/09*

distribution at sex-linked loci in populations where sex reversal occurs.

Evolutionary Consequences

Besides the direct demographic consequences of sex reversal through sex-ratio effects, X–Y recombination should generate bursts of additive genetic variance for male fitness. Let us consider a pair of young sex chromosomes with an incipient nonrecombining segment. On this segment are found a sex-determining locus (with a dominant male-determining allele *Y*), a sex-antagonistic locus (with allele *R* favored in males and linked to *Y*), and a functional locus (with a deleterious allele—fixed on the nonrecombining segment as a result of its incipient decay). In the absence of recombination, a XY male will produce only two kinds of gametes, Y^{R-} and X^{r+}. In contrast, a sex-reversed XY female may produce eight different kinds of oocytes, namely X^{r+}, X^{r-}, X^{R+}, X^{R-}, Y^{r+}, Y^{r-}, Y^{R+}, and Y^{R-}. When mated with a normal male, this female will thus produce a diversity of offspring of highly variable qualities. Among them will appear $Y^{R+}X^{r+}$ "supermales," with a recombinant Y chromosome from their mother containing the male-specific sex-antagonistic allele *R*, but not the deleterious mutation. There will also be, however, low-quality males, such as $Y^{R-}Y^{r-}$, inheriting two copies of the deleterious mutation. It is worth recalling here that the several YY *H. japonica* males found by Kawamura and Nishioka (1977) were albinos, and thus presumably homozygote for a deleterious mutation of a coloration gene on the sex chromosome.

Because the new haplotypes generated by sex reversal do not recombine in males, the best ones (i.e., those without deleterious mutations) should be quickly fixed by natural or sexual selection. Occasional sex reversal may thus contribute to fuel the genetic variance in male fitness required for the evolutionary maintenance of female choice, but only shortly so. Relevance for good-genes models of sexual selection is thus certainly limited, but evolutionary consequences should be long lasting. The fittest haplotypes should rapidly invade populations via selective sweeps, homogenizing sex chromosomes over large geographic ranges. By purging the deleterious mutation load that normally accumulates in nonrecombining Y chromosomes, sex reversal may thus counteract Muller's ratchet, rejuvenating sex chromosomes on a regular basis.

How the frequency of such recombination events affects the dynamics of deleterious- and sex-antagonistic mutations would deserve a proper formalization. Sex-antagonistic genes induce a direct and immediate selective pressure for lowered recombination, but the ensuing accumulation of deleterious mutations will progressively build up evolutionary benefits for an occasional recombination. This raises the intriguing possibility that sex-reversal might be favored (provided it is rare), and thus be more than the mere side-product of a physiological dysfunction.

Empirical Perspectives

Direct evidence for the processes outlined here should come from extensive genetic analyses of allelic frequencies and sex-specific recombination patterns at sex-linked loci during an episode of sex reversal, combined with anatomical assignment of phenotypic sex, along the line exemplified by Matsuba et al. (2008). Research should focus on those fish and lizard species that have been shown to display mixed sex-determination systems, as well as on rare events of sex reversal in species where sex is normally determined genetically.

Indirect evidence might also be gained from the genetic signature of passed sex-reversal and X–Y recombination events. How can we identify groups in which such processes might have played a role? The quest should focus on taxonomic groups (at the level of genus or species group) in which taxa have diverged long enough for autosomal genes to show significant differentiation, but sharing the same homomorphic sex chromosomes (i.e., the same sex-linked markers) with no male recombination. Good candidates for such comparisons are certainly to be found in fish and amphibians. Within anurans, recent findings on European treefrogs (*Hyla arborea* group) point to this group as a promising candidate (Appendix).

In such groups, X and Y chromosomes should be compared both within and among species. Within species, population-genetic studies and sibship analyses should ask whether the same markers can be amplified from both the X and the Y (pointing to a close similarity in primer sequences), whether these markers show recombination in females but not in males, and whether allelic distributions on X and Y copies overlap, despite nonrecombination in males. Among species, phylogenetic studies should ask whether sequences from the Y chromosome of any given species show greater similarity with the homologous sequences on the X chromosome of the same species, than with that of the Y chromosome of sister species sharing the same nonrecombining segment. Such comparisons should be performed on a variety of sequences widely distributed along the nonrecombining segment, because of possible confounding signals from local gene conversion events (Pecon Slattery et al. 2000), or from genes that were recently and independently incorporated into an expanding nonrecombining segment. From the present hypothesis, a strong homology between X and Y sequences is predicted all along the nonrecombining region, except for the few genes directly involved in sex determination or sex-antagonistic actions.

Appendix X–Y Recombination in *H. Arborea* Sex Chromosomes?

Like all other Eurasian treefrogs, the European treefrog (*H. arborea*) possesses undifferentiated sex chromosomes (Anderson 1991). When mapping linkage groups through sibship analyses, Berset-Brändli et al. (2008) identified seven sex-linked

microsatellite markers. All of them are nonrecombining in males, which points to an extensive (and thus possibly old) nonrecombining segment. However, these markers also show evidence of recent recombination events: products can be amplified from both the X and the Y chromosomes using the same primers, and with a large overlap in allelic frequencies (Berset-Brändli et al. 2007, 2008). In addition, the X and Y copies of a sex-linked gene (the transcription co-factor MED15) display striking sequence similarities despite absence of recombination in males, with not a single site substitution over ca 2400 sites compared, including >800 synonymous sites (Niculita-Hirzel et al. 2008). The only sex-differences found correspond to frame-preserving indels in polyglutamine chains, which are known for their high rates of mutation by slippage. The X and Y copy presumably fixed different alleles from a shared polymorphism after the last recombination event. Interestingly, this gene appears to be also sex-linked in the related *H. intermedia* and *H. sarda* (Berset-Brändli et al. 2006), which diverged 4–5 My ago from *H. arborea* (Stöck et al. 2008; Verardi et al. 2009), suggesting that male recombination may have stopped >4–5 My ago in this genomic region. With at least eight species or subspecies around the Mediterranean sea (Stöck et al. 2008), the *H. arborea* group thus offers promising opportunities to search for possible indices of X–Y recombination, the more so that spontaneous sex-reversal has been documented in the closely related *H. japonica* (Kawamura and Nishioka 1977).

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