RESEARCH COMMENTARY

Inexorable spread: inexorable death? The fate of neo-XY chromosomes of grasshoppers

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In a recent article, Veltsos *et al.* (2008) proposed a hypothesis for the spread of a newly arisen Y chromosome throughout the range of a species using as a model the alpine acridid grasshopper *Podisma pedestris* that has two chromosomal forms: a standard X0/XX and a neo-XY/neo-XX race, which form a hybrid zone in the Southern Alps (Hewitt 1975). Veltsos *et al.* (2008) made computer simulations based on the premise that, although Y chromosomes have an evolutionary tendency towards erosion and eventual loss (Charlesworth 1978, 2002; Charlesworth and Charlesworth 2000; Charlesworth *et al.* 2005), there is a continuous recycling of Y chromosomes by *de novo* formation and fixation of neo-Y chromosomes. The model clearly explains the possible spread of a new Y chromosome throughout the geographic range of the species (Veltsos *et al.* 2008; Pannell and Pujol 2009), but it does not take into account the evolutionary fate of the neo-chromosomes. We discuss here that, although neo-XY chromosomes of grasshoppers may be a useful model to explain the spread of a new chromosomal rearrangement, they are not a predictive example of sex-chromosome evolution and recycling.

The origin of sex-chromosomes, and the mechanisms through which they influence sex-determination, are biological issues of the utmost relevance for the understanding of a number of evolutionary problems that have been with us since the earliest times of Mendelism and the chromosome theory of heredity (Kingsland 2007; Bidau and Martí 2001; Castillo *et al.* 2010b). It is current theory that sex chromosomes (XY/XX; ZW/ZZ) evolved from a pair of 'normal' (homomorphic) homologous chromosomes (Moore 2009; Traut 2010). The most accepted hypothesis is that once

a fundamental sex-determination mutational change occurs in a member of an autosomal pair, sex-chromosome evolution starts; in fact, sex chromosomes never stop evolving (Carvalho 2002; Charlesworth and Charlesworth 2005). However, as Charlesworth *et al.* (2005) indicate, it is central that two separate mutations arise for producing genetically distinct males and females. Then, restriction of intrachromosomal recombination (crossing over) between both homologues may be selected for (Charlesworth 2002). Suppression of crossing over may initially be achieved through a heterozygous chromosomal rearrangement (e.g., an inversion; Ohno 1967) which may lead to divergence between both homologous chromosomes (an indispensable condition for the evolution of chromosomal sex determination), and degeneration of the proto-Y (or proto-W) chromosome. Degeneration may involve further chromosomal rearrangements and accumulation of tandem arrays of repetitive DNA and transposable elements such as retrotransposons which could remodel former euchromatic structures into heterochromatic ones (Charlesworth *et al.* 2005; Steinemann and Steinemman 2005). Muller's ratchet, the continuous stochastic loss of chromosomes carrying the fewest numbers of deleterious mutations, has been proposed as a mechanism promoting Y chromosome degeneration (Charlesworth 1978; Engelstädter 2008; Kaiser and Charlesworth 2010). The Hill-Robertson effect may also cause the overall loss of diversity in Y chromosomes (Charlesworth and Charlesworth 2000; Wilson and Makova 2009). In the Orhtoptera, an X-autosome Robertsonian fusion may automatically create a crossover-free paracentromeric region (Bidau 1990). Further chromosomal rearrangements may follow, increasing the genetic isolation between both sex chromosomes (Bidau and Martí 2001; Castillo *et al.* 2010a,b).

At the molecular level it is known that evolution of sex chromosomes is accompanied by the degeneration of genes on the Y (or W) chromosome and the accumulation of various classes of repetitive DNA sequences in nonrecombining regions (Charlesworth 1991; Hobza *et al.* 2006). Consequently, many theoretical studies focus on the forces that produce the morphological and genetic changes between the differential sex chromosomes; especially on how abolition of recombination starts between a pair of homomorphic homologous chromosomes, and why suppression of crossing over leads to the process of genetic degeneration of the Y (Charlesworth 1996; Charlesworth *et al.* 2005; Nicolas *et al.* 2005; Pannell and Pujol 2009).

The process of progressive degeneration of the Y chromosome poses some intriguing problems (Turner 2005; Pannell and Pujol 2009). There are large groups of insects (and other invertebrates) in which the vast majority of species lack a Y chromosome (e.g. Orthopteroid insects). However, it is always assumed that the ancestors of these taxa had typical XY/XX sex chromosome systems (White 1973; Pannell and Pujol 2009; Kaiser and Bachtrog 2010). Thus, it seems that degeneration of the Y is an inexorable process that eventually leads to its loss, or perhaps to its translocation to autosomes (John and Shaw 1967). However, the evolutionary persistence of Y chromosomes in most groups suggests that they can arise *de novo* (Veltsos *et al.* 2008; Pannell and Pujol 2009). Neo-XY systems suggest that the *de novo* origin of Y chromosomes is what actually happens in the Orthoptera and many other taxa. This situation implies a further problem: how does the new Y chromosome that arose as a spontaneous mutant in a single individual, become fixed in a population and subsequently spread throughout the range of the species? Problems arise in the first place because male carriers of the sexual rearrangement are structural heterozygotes and these may have reduced fertility due to meiotic segregation difficulties, or altered recombination patterns thus requiring, for example, chance fixation in a small population by drift. However, this does not explain further spread throughout the species' range because the new form will represent a minority when meeting the ancestral form. Veltsos *et al.* (2008) have modelled this problem in the hybrid zone between the standard X0/XX and the neo-XY/neo-XX races of the alpine grasshopper *P. pedestris* (Hewitt 1975). Veltsos *et al.* (2008) have found that sexually antagonistic effects of the neo-Y induce indirect selection in favour of the fused X-chromosomes resulting in their spread in the hybrid zone. The neo-Y thus can spread because it is protected behind the advancing shield of the neo-X distribution despite having mutations (accumulated because of restrictions on recombination with the neo-X) that could cause it to be selected against. It seems paradoxical that its degeneration can be the factor that favours its spread (Pannell and Pujol 2009).

Although this model is very likely to be correct, it ignores a problem that may render the idea of sex-chromosome recycling in Orthoptera useless. It is puzzling how neo-Y species of Orthoptera do not revert to an XO state by erosion of the neo-Y chromosome. Evolutionarily recent neo-XY systems are characterized by full pachytene synapsis of neo-Y and XR (the neo-X counterpart of the original autosome involved in the centric fusion that originated the new sex chromosomes), interstitial chiasma formation, and lack of heterochromatinization of the Y as in *P. pedestris* (Hewitt 1975), *Hesperotetix pratensis* (McClung 1917), *Hypochlora alba* (King 1950), (Bugrov and Grozeva 1998) *Baeacris punctulatus* (Castillo *et al.* 2010b), and *Leiotettix sanguineus* (Mesa and de Mesa 1967), while advanced or 'old' systems show restriction of synapsis and recombination between both elements, which only maintain a distal-terminal association for proper meiotic segregation (e.g. *Oedaleonotus enigma*; Hewitt and Schroeter (1968), *Stenobothhrus rubicundus* (John and Hewitt 1968), and *Ronderosia ommexechoides* (Carbonell and Mesa 2006)). In these cases, the neo-Y is usually almost completely heterochromatic and sometimes, structurally rearranged (both, signs of degeneration) as in *Ronderosia bergi* (Castillo *et al.* 2010a), and in some cases, the XR arm has also became heterochromatinized as in *Aleuas* and *Zygoclistron* species (Mesa *et al.* 2001). In extreme cases, even XL (the original X chromosome) may be involved in a complex internal chromosomal rearrangement as in *Dichroplus vittatus* (Bidau and Martí 2001). Intermediate cases exhibit a mixture of characteristics of both ends of this continuum (Castillo *et al.* 2010b). Indeed, the idea of orthopteran neo-Y degeneration was implicit in a classic paper by Sáez (1963) where heterochromatinization of the neo-Y was comparatively analysed in a wide range of South American and North American acridoid species.

It is thus puzzling that not a single instance of complete loss of the neo-Y has been observed in the Orthoptera, which would be expected as the final stage of Y chromosome degeneration. Evolutionary loss of the Y from a former neo-XY system would not be difficult to detect owing to the special morphological characteristics of the neo-X chromosome as compared with the standard X chromosome. If orthopteran neo-Y chromosomes were indeed lost after the degeneration process it is not implausible that, in the initial steps of elimination it could remain as a B chromosome, a situation proposed for supernumerary chromosomes of tsetse flies (Amos and Dover 1981). The relationship of B to Y chromosomes is important since, in some insects, new Y chromosomes are thought to have derived from B chromosomes although this hypothesis awaits further validation (Nokkala *et al.* 2003; Carvalho *et al.* 2009).

Further, in acrids, neo-XY systems have repeatedly and independently evolved towards more complex systems of the $X_1X_2Y/X_1X_1X_2X_2$ kind, through fusion of the neo-Y with a second autosome (Mesa and de Mesa 1967; Castillo *et al.* 2010a,b). That is, the degeneration process might be 'interrupted' by a new cycle of genomic rearrangement. Again, however, not a single case of reversion of X_1X_2Y systems, or loss of the new Y has been observed. The former is somehow expected because loss of the 'neo-neo-Y' would imply a substantial portion of the genome, and males would be left

with two very different X chromosomes that would have to segregate jointly in meiosis to avoid gametic imbalance.

Thus, while orthopteran neo-XY systems are excellent models for the analysis of the spread of a newly arisen chromosomal rearrangement (Veltsos *et al.* 2008; Pannell and Pujol 2009), especially at hybrid zones, they have not given us, until now, any insight on the problems of Y-chromosome degeneration and disappearance, nor on the recycling of sex chromosomes. Are neo-sex systems of Orhtoptera a dead end of evolution? Indeed, Mesa *et al.* (2001) proposed that neo-XY systems lead to extinction without descent (speciation) of species harbouring them. Their main argument was that no major taxa of Orthoptera higher than genus are known to share neo-sex chromosome systems of common origin. And this is true with the possible exception of the Neotropical Aleuasini tribe (Copiocerinae) where all species of the two genera included in the tribe (*Aleuas* and *Zygoclistron*) are neo-XY/neo-XX possibly inherited from a common ancestor (Mesa *et al.* 2001; Castillo *et al.* 2010b), and of the genera *Hesperottetix* and *Mermiria* from the northern hemisphere (White 1973; Castillo *et al.* 2010b), *Tolgadia* from Australia (John and Freeman 1975), and the pamphagid genus *Asiomethis* (Li *et al.* 2005).

Given that although nearly all degrees of genetic degeneration of the Y and progressive elimination of recombination of the neo-sex chromosomes are known in the Orthoptera, it is puzzling that in no case the neo-Y chromosomes have dissapeared, and it is possible that neo-sex chromosomes of Orthoptera have, or acquire, some new property or function that prevents their loss, which is reinforced by the frequent independent evolution of more complex sex systems from the original neo-sex chromosomes, making the loss of the Y even less likely. It is difficult to envisage mechanisms through which new Ys acquire essential sex determining or male fertility genes that would preclude their total elimination because these systems have repeatedly and independently appeared in different genera and families of grasshoppers, katydids and crickets, and each case followed a separate evolutionary history. Indeed, in the production of a neo-sex system, the X chromosome may fuse with almost any autosome of the complement (although large-sized and medium-sized autosomes are more frequently involved; Castillo *et al.* (2010b). However, without the precondition of acquisition of sex-related genes, we would not expect abolition of recombination and degeneration (Charlesworth *et al.* 2005). A further cause for the persistence of orthopteran neo-Y chromosomes despite erosion, could be mechanical: the neo-Y would be essential for disjunctional segregation of the neo-X in male meiosis.

Nevertheless, all the former considerations apply if neo-sex chromosomes of Orthoptera follow the canonical path of sex chromosome evolution. This is not necessarily so: recently, it has been demonstrated that at least in the Diptera, the origin of Y chromosomes may follow quite different paths, and in some cases, Y chromosome evolution may imply the acquisition of new genes and not only gene loss

which has traditionally been considered important in Y chromosome evolution (Koerich *et al.* 2008; Carvalho *et al.* 2009). In fact, Koerich *et al.* (2008) demonstrated that in species of *Drosophila*, the rate of gene gain in the investigated Y chromosomes has been 10.9 times higher than the rate of gene loss, suggesting a prominent role in Y chromosome evolution. This findings contrast with the situation of mammalian Y chromosomes whose gene content consists mainly of relic subsets of X-linked genes which have been differentially lost although a few acquisitions occurred (Graves 2006; Koerich *et al.* 2008; Carvalho *et al.* 2009). It is thus possible that the orthopteran neo-sex chromosomes history may be also noncanonical and that their persistence in very diverse lineages despite degeneration, could be the result of several until now unknown factors, such as gene content of the original autosomes and problems derived from dosage compensation, acquisition of new genes from autosomes as in *Drosophila*, and essential male meiotic mechanical properties.

Despite many descriptive studies of neo-XY chromosomes of Orthoptera, including morphology and meiotic behaviour, nothing is known about their meaning in evolutionary terms and their role in sex determination that, in Orthoptera, remains a mystery (Verhulst *et al.* 2010). These days, a battery of molecular techniques is at the disposal of chromosome researchers. In principle, neo-Y and XR sequences could be compared using chromosome painting, analyses of selected DNA sequences, or in the future more directly, microdissecting both chromosomes, cloning the amplified DNA, and comparing homologous sequences of neo-Y and XR to look for degeneration at first, comparing known repetitive sequences (e.g. 45S rDNA, 5S rDNA, histone genes, etc.), and although more difficult, single copy genes. All these could be done in recent and ancient neo-XY systems, which are available. Neo-XY systems of acridid grasshoppers are a fine and mysterious model to study sex chromosome evolution and Y chromosome degeneration, and deserve attention in this respect.

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