Homomorphic Sex Chromosomes and the Intriguing Y Chromosome of *Ctenomys* Rodent Species (Rodentia, Ctenomyidae)

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Key Words

Ctenomys maulinus · FISH · Meiosis · Microdissection · Sex chromosomes · Tuco-tucos · XY differentiation

light of the regular purge that counteracts Muller's ratchet and the probable mechanisms accounting for their origin and molecular homology.

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Abstract

Unlike the X chromosome, the mammalian Y chromosome undergoes evolutionary decay resulting in small size. This sex chromosomal heteromorphism, observed in most species of the fossorial rodent *Ctenomys*, contrasts with the medium-sized, homomorphic acrocentric sex chromosomes of closely related *C. maulinus* and *C. sp.* To characterize the sequence composition of these chromosomes, fluorescent banding, self-genomic in situ hybridization, and fluorescent in situ hybridization with an X painting probe were performed on mitotic and meiotic plates. High molecular homology between the sex chromosomes was detected on mitotic material as well as on meiotic plates immunodetected with anti-SYCP3 and anti-γH2AX. The Y chromosome is euchromatic, poor in repetitive sequences and differs from the X by the loss of a block of pericentromeric chromatin. Inferred from the G-banding pattern, an inversion and the concomitant prevention of recombination in a large asynaptic region seems to be crucial for meiotic X chromosome inactivation. These peculiar findings together with the homomorphism of *Ctenomys* sex chromosomes are discussed in the

The evolutionary heteromorphism in mammalian sex chromosomes has attracted the attention of biologists since Muller's [1914] proposition of their autosomal derivation and subsequent acquisition of the testis-determining factor (nowadays known as *SRY* in mammals) [Harley et al., 1992, 2003]. As a result, partial or complete suppression of crossing-over between the heteromorphic sex chromosomes (XY or ZW) and functional degeneration of the Y (or W) has been reported previously [Charlesworth and Charlesworth, 2000; Charlesworth et al., 2005; Ellegren, 2011]. To explain these phenomena, 2 models have been proposed [Jablonka and Lamb, 1990] and discussed elsewhere [Ohno, 1969; Charlesworth, 1991; Graves, 1995, 2006; Charlesworth and Charlesworth, 2000]. The 'conformational' model assumes that chromatin modification affecting the sex-determining genes is a triggering factor for the heteromorphism. The 'structural' model hypothesizes that inversions or translocations account for the heteromorphism and subsequent crossing-over suppression. Noteworthy, the functional and long-

term consequences of this heteromorphism, i.e. meiotic sex chromosome inactivation (MSCI) [Turner, 2007; Burgoyne et al., 2009] and dosage compensation, are not fully understood [Heard and Disteche, 2006; Chow and Heard, 2009; Disteche, 2012; Livernois et al., 2012].

Muller [1914, 1918] suggested that without crossing-over, the Y chromosome would accumulate deleterious recessive mutations based on the impossibility of such mutations to become homozygous. Consequently, absence of recombination would result in Y degradation, exacerbated by genetic drift (Muller's ratchet) and inefficient selection [Nei, 1969; Charlesworth and Charlesworth, 1980; Bull, 1983; Rice, 1987; Charlesworth, 1991]. This drifting effect is inversely correlated to the population size of Ys, whose frequency is only one-fourth of that of the autosomes, generating low selection coefficients in deleterious mutations and accelerating its fixation (background selection) [Charlesworth, 1994, 1996]. Selection inefficiency also decreases the Y's ability to fix favorable mutations [Orr and Kim, 1998], while it increases the fixation of deleterious ones by hitchhiking among completely linked loci (Hill-Robertson interference) [Rice, 1987; Charlesworth and Charlesworth, 2000]. This process is further exacerbated by the Y's high mutation rate derived from higher cell division rates in the testis relative to the ovaries [Li et al., 2002]. In short, Muller's ratchet, background selection, the Hill-Robertson effect with weak selection, and the hitchhiking of deleterious alleles by favorable mutations have been listed to explain the decay of the Y chromosome [Charlesworth and Charlesworth, 2000].

Although the structure and composition of the mammalian sex chromosomes has been backed up by empirical studies [Skaletsky et al., 2003; Ross et al., 2005], the evolution of sex chromosomes has not been fully addressed due to the lack of data from suitable organisms. In this respect, homomorphic sex chromosomes represent an excellent possibility to test the predictions concerning their differentiation, including the degradation process [Charlesworth et al., 2005; Graves, 2006; Bergero and Charlesworth, 2009; Wilson and Makova, 2009; Bachtrog, 2013].

The fossorial genus *Ctenomys* represents a speciose group of the South American mammal radiation, exemplified by the description of more than 70 nominal species [Reig and Kiblicky, 1969; Reig et al., 1990; Lessa and Cook, 1998; Wood and Kilpatrick, 2005]. This radiation is associated with a rather conservative genome size [Ruedas et al., 1993; Gallardo et al., 2003], extremely high rate of chromosomal change [Reig and Kiblicky, 1969; Gal-

lardo, 1979, 1991; Reig et al., 1990; Ortells, 1995; Gallardo et al., 2003; Parededa and Novello, 2012], and recurrent amplifications and deletions of satellite DNA [Rossi et al., 1990; Slamovits et al., 2001].

Two Andean species, *C. maulinus* ($2n = 26$, $FN = 48$), and *C. sp.* ($2n = 28$, $FN = 50$) share mono-armed, medium-sized, and homomorphic sex chromosomes [Gallardo, 1979, 1991]. This represents a derived trait considering that most species have distinctively biarmed, heteromorphic sex chromosomes [Reig and Kiblicky, 1969; Gallardo, 1979, 1991; Reig et al., 1990; Ortells, 1995; Parededa and Novello, 2012].

Aiming to shed some light on the chromatin constitution, mapping, origin, and evolution of the homomorphic sex chromosomes of these *Ctenomys* species, we evaluated their molecular composition through an array of cytogenetic techniques. To this end, we conducted fluorescent in situ hybridization (FISH) using an X-specific probe on mitotic and meiotic chromosomes, self-genomic in situ hybridization (self-GISH), C- and fluorescent banding to map and estimate the relative amount of the sex chromatin, as well as immunofluorescence-FISH to explore the sex body's meiotic behavior.

Material and Methods

Chromosomes

Mitotic plates of 2 males and 2 females of each *C. maulinus* and *C. sp.*, parapatrically distributed in Lonquimay, Malleco, Chile ($38^{\circ}25'S$, $71^{\circ}30'W$), were analyzed. Chromosomal material was obtained from primary fibroblast cultures derived from lung tissue, following Verma and Babu [1995]. Meiotic spreads from 1 male of each species were obtained according to Peters et al. [1997]. Briefly, a testicular cell suspension in 100 mM sucrose was spread onto slides. Slides were previously dipped in a solution containing 1% paraformaldehyde and 0.15% Triton X-100 dissolved in distilled water. Subsequently, they were allowed to dry for 2 h in a moist chamber, washed with 0.04% Photo-flo solution (Kodak), air-dried, and rehydrated in PBS.

X Chromosome Microdissection and Amplification

X chromosomes, representing the only acrocentrics of the female karyotype of *C. maulinus* [Gallardo, 1979, 1991] (online suppl. fig. 1; see www.karger.com/doi/10.1159/000366173 for all online suppl. material), were microdissected with an Eppendorf TransferMan NK2 micromanipulator coupled to a Zeiss Axiovert 200 microscope. Since the homomorphism of the XY pair poses a potential risk of misidentification, males were not used for microdissection of sex chromosomes. Males of the allied octodontid *Spalacopus cyanus* were used as an external control. About 15–20 chromosomes were separately placed in 9 μ l DNase-free ultrapure water and amplified by the GenomePlex Single Cell Whole Genome Amplification Kit (WGA4, Sigma-Aldrich) [Gribble et al., 2004]. Amplified samples (300–600 bp) were labeled with the Ge-

Fig. 1. Female (**a, b**) and male (**c, d**) mitotic metaphases of *C. maulinus* (**a, c**) and *C. sp.* (**b, d**) after FISH with the *C. maulinus* X chromosome probe. Details of X and Y chromosomes are shown by the separation of color channels underneath each figure. White arrows in **c** and **d** indicate the Ys' pointed pericentromeric regions that allow their identification in both species.

nomePlex Reamplification Kit (WGA3, Sigma-Aldrich), using digoxigenin-11-dUTP (Roche Applied Science), following Gribble et al. [2004].

FISH with the X Chromosome Probe and Barr Body Counting

Slides containing chromosomes and interphase nuclei of both species were hybridized with the whole X chromosome probe of *C. maulinus* following the procedure of Pinkel et al. [1986]. Hybridization signals were detected with anti-digoxigenin-rhodamine (Roche Applied Science). After 3 washes in 4× SSC/0.1% Tween 20 for 5 min, slides were counterstained with DAPI and mounted with antifade solution (Vectashield). Mitotic plates were digitally captured with an Axiolab epifluorescence microscope (Carl Zeiss) equipped with an Axiocam camera and adequate filters. Images were overlaid and contrast-enhanced using Adobe Photoshop CS6.

To count Barr bodies in interphase nuclei, slides were dismounted, washed 3 times in 4× SSC/0.1% Tween 20 for 5 min and stained with orcein [Verma and Babu, 1995]. By combining fluorescence emission with halogen light, Barr body counting was performed in 300 nuclei of each individual analyzed, totaling 1,200 nuclei.

Self-GISH and Telomere FISH

Highly repetitive sequences of each *Ctenomys* species were detected by hybridizing their total genomic DNA onto their own mitotic plates, as described by She et al. [2007] and modified by Suárez-Villota et al. [2012]. Telomere detection by FISH, carried out on metaphase chromosomes, followed Moyzis et al. [1988]. The (TTAGGG)_n probes were PCR-generated and labeled with fluorescein-12-dUTP (Roche Applied Science) [Ijdo et al., 1991]. Images were captured as described above.

Chromosome Banding

C-banding was done according to Sumner [1972]. G-banding was conducted according to Seabright [1971], with slight modifications. Slides were treated with 0.025% trypsin (Gifco 1:250) dissolved in Ca²⁺- and Mg²⁺-free Hanks' solution at pH 5.5 for about 105 s, rinsed in alcohol (90 and 100%), and air-dried. Chromosomes were stained with 5% Giemsa dissolved in phosphate buffer (pH 6.8).

To visualize blocks of AT-rich sequences, methyl-green/DAPI staining was performed [Donlon and Magenis, 1983], whereas CG-rich chromatin regions were identified through chromomycin

Fig. 2. Female (**a**, **c**) and male (**b**, **d**) interphase nuclei of *C. maulinus* after FISH with the X chromosome probe of *C. maulinus* (**a**, **b**) and orcein staining (**c**, **d**). The same signal pattern per sex was observed in interphase nuclei of *C. sp.*

Fig. 3. Sex chromosomes of *C. maulinus*. **a** Self-GISH. **b** FISH using the universal telomeric probe. A white arrow indicates the pericentromeric region of the X chromosome, undetected by the DAPI staining. **c** CMA₃ banding. **d** Methyl-green/DAPI banding. **e** C-banding. **f** G-banding. The probable location of the inversion breakpoints is indicated (see text for details).

(CMA₃) staining [Schweizer, 1980]. Using a halogen lamp (for C- and G-banding) and a mercury lamp with adequate filters (for chromomycin and methyl-green/DAPI staining), specific bands were observed. Banding images were captured at 100× with an AxioLab epifluorescence microscope (Carl Zeiss) equipped with an AxioCam camera.

Immunofluorescence-FISH on Meiotic Spreads

Meiotic spreads of both *Ctenomys* species were vaporized with distilled water for 15 min and blocked with 4% BSA for 10 min. Spreads were subsequently incubated in a moist chamber at 4°C for 24 h. The incubation solution contained rabbit antibodies against the synaptonemal complex protein 3 (SYCP3; Abcam, Cat. No. 15093) diluted 1:50 in PBS, and mouse monoclonal antibody against histone H2AX phosphorylated at serine 139 (γH2AX; Upstate, Cat. No. 05-636) diluted 1:3,000 in PBS. After rinsing in PBS, slides were incubated in a moist chamber with donkey anti-rabbit IgG conjugated with fluorescein isothiocyanate (FITC; Jackson Immuno Research Laboratories) for 1 h at room temperature. The solution for the second incubation also contained goat anti-mouse IgG conjugated with Alexa Fluor 405 (Invitrogen, Cat. No. A-31553) diluted 1:100 in PBS. Slides were subsequently washed in 2× SSC for 5 min, dehydrated in an ascending ethanol series (50, 70, and 99%) and denatured in 60% formamide for 3 min at 65°C. Then, they were washed in 2× SSC and dehydrated in an ascending ethanol series for 3 min before hybridization. The same hybridization mixture and post-hybridization conditions of FISH were used for immunodetected meiotic spreads. Images were captured and treated as described previously.

Results

FISH with X Chromosome-Specific Probe and Barr Body Counting

The X chromosomes of the females of both *Ctenomys* species were clearly detected by the *C. maulinus* X probe in mitotic plates (fig. 1a, b). Interestingly, the probe hybridized to both the X and Y chromosomes in male metaphases (fig. 1c, d). The pericentromeric region of the Y is more pointed than that of the X (see white arrows in fig. 1c, d), allowing their discrimination in both species (fig. 1a–d, bottom panels). This pattern was clearly observed in 270 of the 300 metaphases analyzed. Control assays using the X probe on male mitotic plates of the related octodontid, *S. cyanus*, detected only 1 chromosome, as expected (online suppl. fig. 2). Similar to metaphases, sex chromosomes were detectable by FISH in male and female interphase nuclei of both species (fig. 2a, b). Only 1 Barr body was detected in 83% of interphase female nuclei, and none was detected in 77% of male nuclei (fig. 2c, d), as expected.

Cytogenetic Characterization of the Sex Chromosomes

A large pericentromeric region of highly repetitive sequences was detected by self-GISH on the X chromosome of *C. maulinus* (fig. 3a), whereas an interstitial block of repetitive chromatin as well as telomeric/subtelomeric signals were detected on the Y chromosome (fig. 3a). The telomeric probe detected discrete signals at both the pericentromeric and the telomeric region of *C. maulinus* sex chromosomes (fig. 3b).

Fig. 4. Meiotic plates of *Ctenomys* species. **a** SYCP3 immunodetection at early pachytene of *C. sp.* allowed the identification of the PAR and the asynaptic region of the sex bivalent (left). The γ H2AX histone variant colocalized with the X probe signal only in the asynaptic region (compare center and right panels). **b** Late pachytene of *C. maulinus* immunodetected with SYCP3 (green, left), and hybridized with the X chromosome probe (center). The double immunodetection of γ H2AX and SYCP3 reveals the colocalization of this histone variant with the sex chromosome pair (right).

A large block of GC-rich repetitive pericentromeric sequences was detected by CMA₃ on the X chromosome of *C. maulinus* (fig. 3c). The rest of the X as well as the Y chromosome were AT-rich, as evidenced by the methyl-green/DAPI banding (fig. 3d). Interestingly, the distance between the second and the third band on the Y chromosome is longer than that on the X chromosome as evidenced by the AT-rich banding procedure (fig. 3d, white arrow).

The C-banding of the X chromosome comprised a large block of pericentromeric heterochromatin on *C. maulinus*, whereas a slightly-stained interstitial band was observed in its Y chromosome (fig. 3e). Since the X probe signal colocalized with the DAPI staining (fig. 1, bottom panels) and since DAPI staining did not detect the pericentromeric heterochromatin of the X (fig. 3b, white arrow), this region was neither detected by the X probe nor by the methyl-green/DAPI staining (fig. 3d).

Four distinctive, evenly distributed dark G-bands not reaching the repetitive pericentromeric block were detected on the X chromosomes of *C. maulinus* (fig. 3f). Similarly, 4 dark bands forming 2 groups of 2 bands separated by a large light-stained interstitial region were detected on its Y chromosome (fig. 3f). In short, these sex chromosomes share similar patterns of repetitive sequences and C- and G-banding patterns in both species (fig. 3, online suppl. fig. 3).

Immunofluorescence-FISH on Meiotic Spreads

Fourteen bivalents were detected in the spermatocytes of *Ctenomys* sp. (fig. 4a), whereas those of *C. maulinus* exhibited 13 bivalents (fig. 4b). A small pseudoautosomal region (PAR) was observed in the sex bivalent of *C. sp.* during early pachytene. Moreover, the axial element of its Y chromosome was shorter than that of the X (fig. 4a, left). The X probe recognized the whole sex body of *C. sp.* by early pachytene (fig. 4a, center), whereas the γ H2AX signal distribution was restricted to its asynaptic region (fig. 4a, right). Both species shared these characteristics during early pachytene.

The sex bivalent detected by SYCP3 was easily recognized by its strong green signal and secondary modifications of the synaptonemal complex at late pachytene in both species (fig. 4b, left). At this stage, the X chromosome probe recognized the whole sex body (fig. 4b, center). The colocalization of its signal with that of the γ H2AX histone variant corroborated the identification of the sex chromosomes (compare center and right pictures of fig. 4b).

Discussion

Phylogenetic analyses using cytochrome *b* sequences and copy number of the major satellite DNA showed that *C. maulinus* forms a monophyletic, derived clade relative to *C. talarum*, *C. mendocinus*, *C. rionegrensis*, *C.*

Fig. 5. Maximum-likelihood tree for the ctenomyines based on Slamovits et al. [2001]. Sex chromosome images beside the species names were taken from the following references: 1 = Gallardo [1991]; 2 = Cook and Salazar-Bravo [2004]; 3 = Anderson et al. [1987]; 4 = Kelt and Gallardo [1994]; 5 = Ortells et al. [1990]; 6 = Reig and Kiblicky [1969]; 7 = Parededa and Novello [2012]; 8 = Massarini et al. [1991a]; 9 = Massarini et al. [1991b]. Note the prevalence of heteromorphic sex chromosomes in Ctenomyidae and the derived condition of homomorphic sex chromosomes in *C. maulinus* (framed).

australis, *C. porteousi*, and *C. pearsoni* (fig. 5) [Slamovits et al., 2001]. These, and most other species, share distinctively biarmed, heteromorphic sex chromosomes with a small, totally heterochromatic metacentric to subtelocentric Y (fig. 5). Nevertheless, the acrocentric Y

of the species studied here is medium-sized and largely euchromatic (fig. 3e, online suppl. fig. 3e). Contrary to most mammals [Moruzzi, 1979; Waters et al., 2007], the Y represents 6–7% of the linear haploid female set [Gallardo, 1979].

The outgroup and commonality criteria [Hennig, 1966] used to reconstruct the phylogenetic relationships within the Ctenomyidae (fig. 5) allow to infer that monoarmed, medium-sized sex chromosomes represent a derived condition, attributed to a pericentric inversion from a biarmed state [Gallardo, 1991]. Additional explanatory mechanisms are needed to account for the large size and absence of heterochromatin of the Y. Among them, chromatin duplication or its translocation to the Y, and epigenetic modifications like the euchromatinization of heterochromatin [King, 1993; Grewal and Jia, 2007], have been suggested. Moreover, the homomorphism and the high molecular homology of the sex chromosomes (fig. 1) require complex, explanatory cytogenetic mechanisms. Among them: (A) Sex chromosome turnover resulting in homomorphic XY chromosomes with high molecular homology has been proposed. This hypothesis requires new autosomal master sex-determining genes to replace previous sex chromosomes before the accumulation of deleterious mutations or structural changes [Schartl, 2004; Volff et al., 2007]. Although this turnover has been reported in several congeneric fishes [Kondo et al., 2004; Peichel et al., 2004; Volff et al., 2007; Baroiller et al., 2009; Yoshida et al., 2014] and amphibians [Hillis and Green, 1990; Miura, 2007], its applicability to our case is difficult to sustain when using more distantly related, non-congeneric taxa (online suppl. fig. 1). (B) Local gene conversion with occasional genetic exchanges within the non-recombining regions of the X and Y chromosomes [Slattery et al., 2000]. The restrictive nature of gene conversion renders this mechanism unsuitable due to the massive exchanges in the asynaptic region needed to account for the large-scale X-Y similarity depicted in figure 1c, d. (C) Sex chromosome recombination in sex-reversed XY females. Since sex (and not the genotype) is the primary determinant of meiotic recombination in mammals, the rate and pattern of recombination in XY mouse oocytes is almost identical to that in XX oocytes [Lynn et al., 2005; Perrin, 2009]. Rare events of recombination in XY females can prevent the decay of Y chromosomes, as discussed elsewhere [Perrin, 2009; Stöck et al., 2011]. Although functional XY females are rare in mammals, and deviations in the sex chromosome constitution typically result in

sterility [Orr, 1990; Vaiman and Pailhoux, 2000], fertile XY sex-reversed females have been reported in *Myopus schisticolor* [Fredga et al., 1976], *Dicrostonyx torquatus* [Fredga, 1988], *Microtus cabreræ* [Burgos et al., 1988], and in 9 *Akodon* species [Bianchi, 2002]. But, had this recombination occurred in *Ctenomys*, FISH would have detected the signature of segments translocated to the Y. Likewise, if a non-reciprocal translocation from the X to the Y had occurred, segments of the original Y would not be detected by the X probe. Contrary to the expectations, the X probe recognizes the whole Y chromosome (fig. 1c, d). (D) Recent fusion of an autosome to the ancestral X/Y pair. This hypothesis implies that the X probe should be also detected in autosomes in related species, but not in the original Y of *Ctenomys*. None of these predictions were fulfilled by our empirical test (fig. 1, online suppl. fig. 3). (E) Sex reversal by translocation of the *SRY* gene to the X chromosome resulting in a neo-Y through local recombination (option B), recombinant XY events in the oocytes (option C) or by any other mechanisms that transfer or interchange non-homologous segments between the sex chromosomes (e.g. transposition, reciprocal and non-reciprocal translocations). In fact, *SRY* translocations are frequent in humans [Auwera et al., 1992; Margarit et al., 2000; Zenteno-Ruiz et al., 2001] and rodents [Koopman et al., 1991; Jiménez et al., 2013]. Since the eutherian *SRY* gene ranges in size from 700 to 2,000 bp [Goodfellow and Lovell-Badge, 1993], FISH based on whole chromosome probes would not be sensitive enough to detect such an event [Schubert et al., 2001; Sharma and Sharma, 2001]. Accordingly, the *SRY*-bearing X might not be totally detected by the X chromosome probe, as observed here (fig. 1). This neo-Y model in *Ctenomys* would explain in 1 step both the high molecular homology between the sex chromosomes and the evolutionary transition involving size increase, euchromatinization, and chromosomal transformation into a monoarmed Y. Nevertheless complete sequencing of the sex chromosomes of *Ctenomys* and its comparison with the genomes of related species will be needed to discern among these 5 alternatives.

As the sex chromosomes of these species share similarities in morphology, size, banding patterns, and molecular homology their undifferentiated stage is suggested (fig. 1c, d and fig. 3d, f). Theoretically, partial or total absence of recombination in these proto-sex chromosomes may lead to their genetic divergence and ultimately to Y chromosome decay [Muller, 1914; Charlesworth et al., 2005]. One way in which this ces-

sation could come about is through inversions or other rearrangements involving the sex-determining locus. This would impose a direct hindrance to crossing-over between the proto-sex chromosomes [Jablonka and Lamb, 1990; Charlesworth et al., 2005; Bergero and Charlesworth, 2009]. In this regard, G- and C-band comparison (fig. 3e, f, marked area) as well as the different distance of interstitial AT-rich bands in X and Y (fig. 3d, white arrow) allow us to infer that a paracentric inversion affected these sex chromosomes. Probably, since its onset, this rearrangement resulted in chromosomal asynapsis and other impairments in heterozygotes [Burgoyne et al., 2009; Lemaitre et al., 2009]. Congruent with such a putative scenario, a large asynaptic region between the X and Y is observed in early pachytene (fig. 4a). Contrasting with the PAR (fig. 4a, left), this asynaptic part exhibited γ H2AX accumulation as an evidence of cessation of recombination and MSCI [Plug et al., 1998; Turner et al., 2004; Turner, 2007; Burgoyne et al., 2009]. Asynapsed regions at pachytene exhibit persistence of unrepaired double-strand breaks (DSBs) due to loss of repair by homologous recombination [Plug et al., 1998; Turner, 2007]. Consequently, repair proteins already recruited by the DSBs remain in the asynapsed X and Y axes, which in turn recruits additional ATR kinase. ATR then spreads into the chromatin loops and phosphorylates H2AX, triggering the chromatin changes that lead to MSCI and sex body formation [Turner et al., 2004; Burgoyne et al., 2009].

Once suppression of recombination is achieved, the genetic degeneration of the Y and the X dosage compensation mechanism would start [Charlesworth and Charlesworth, 2000; Charlesworth et al., 2005]. Evidence for the degeneration of the initial Y chromosome is exemplified by the loss of a GC-rich block (fig. 3a, c, f) and by the accumulation of distinctive interstitial repetitive sequences (fig. 3a). Thus, gains and losses of differential repetitive sequences have apparently occurred in the evolution of the Y, although the latter event is needed to reduce its size [Charlesworth and Charlesworth, 2000; Ross et al., 2005; Bachtrog, 2013]. The Y degeneration, supposed to coevolve with dosage compensation [Charlesworth, 1978; Disteche, 2012; Muyle et al., 2012] is in due course here since the Barr body was found only in females (fig. 2). But to fully understand the factors that have shaped these peculiar sex chromosomes, complete sequencing and analyses of gene expression will be needed.

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