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High intrachromosomal similarity of retrotransposon long terminal repeats: Evidence for homogenization by gene conversion on plant sex chromosomes?

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Abstract

Retrotransposons are ubiquitous in the plant genomes and are responsible for their plasticity. Recently, we described a novel family of gypsy-like retrotransposons, named *Retand*, in the dioecious plant *Silene latifolia* possessing evolutionary young sex chromosomes of the mammalian type (XY). Here we have analyzed long terminal repeats (LTRs) of *Retand* that were amplified from laser microdissected X and Y sex chromosomes and autosomes of *S. latifolia*. A majority of X and Y-derived LTRs formed a few separate clades in phylogenetic analysis reflecting their high intrachromosomal similarity. Moreover, the LTRs localized on the Y chromosome were less divergent than the X chromosome-derived or autosomal LTRs. These data can be explained by a homogenization process, such as gene conversion, working more intensively on the Y chromosome.

Keywords: Repetitive DNA; Sex chromosomes; Dioecious plant; Microdissected chromosomes; Genome evolution

1. Introduction

Multigene families and many non-coding repetitive sequences evolve in a concerted fashion which results in their homogenization. One of the mechanisms responsible for concerted evolution is gene conversion (Liao, 2003). Gene conversion is defined as the recombination and DNA repair processes that leads to replacement of one allele by another. It consists in copying of one stretch of DNA into another. Gene conversion is likely to be involved in homogenization of ribosomal DNA arrays (Schlotterer and Tautz, 1994) as well as satellites (Elder and Turner, 1995; Schueler et al., 2001). The rate of homogenization of such repetitive sequences is higher along chromosomal lineages than between different chromosomes (Warburton and Willard, 1995). This is demonstrated, for example, by the alpha satellite on human centromeres, which is primarily homogenized in a chromosome-specific manner (Schueler et al., 2001).

Homogenization of transposable elements, which represent interspersed repeats, is less understood. Most families of mobile elements are classified into several families; some are ancient and others more recent. Elements that are still active in retroposition have a higher degree of sequence similarity, which reflects recent amplification. However, recent studies reported evidence for gene conversion also in transposable elements. The influence of gene conversion was presented in the MITE elements of *A. thaliana* (Le et al., 2000), human *Alu* elements (Roy et al., 2000) and S2 elements of *D. melanogaster* (Maside et al., 2003). Kass et al. (1995) reported a gene conversion event in which one of the oldest *Alu* family members was converted to one of the youngest *Alu* subfamilies, Yb8. Hood et al. (2005) electrophoretically

Abbreviations: FISH, fluorescence *in situ* hybridization; PCR, polymerase chain reaction; bp, base pair(s); dNTP, deoxyribonucleoside triphosphate; DAPI, 4′,6-diamino-2-phenylindole.

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separated chromosomes of *Microbotryum violaceum* and studied the structure of copia and Helitron transposable elements. They showed a statistically significant similarity among copies originated from the same chromosome which is consistent with the gene conversion hypothesis (Hood et al., 2005).

In sexually reproducing organisms the homogenization processes are significantly accelerated by meiotic recombination. Repetitive satellite sequences are more homogenous in insect species that undergo sexual reproduction than those in asexual species (Mantovani, 1998), which indicates that meiotic recombination plays a major role in sequence homogenization. The gene conversion among copies of satellite DNA located on the sex chromosomes was demonstrated for the first time in *Anopheles gambiae* (Krzywinski et al., 2005). Phylogenetic analysis revealed that satellite DNA monomers originating from the Y and X chromosomes formed separate clades. Two possible mechanisms were proposed to explain the Y-linked array expansion in non-recombining Y chromosome in *A. gambiae*. They included unequal sister chromatid exchange (Smith, 1976) and rolling circle replication followed by DNA reintegration into the genome (Okumura et al., 1987).

Recently, we described a new family of gypsy-like retrotransposons, named *Retand* (Kejnovsky et al., 2006). These retrotransposons are transcriptionally active and are highly abundant in *Silene latifolia*, the dioecious plant with heteromorphic sex chromosomes. Here we show that long terminal repeats (LTRs) of the *Retand* retrotransposons isolated from laser microdissected X and Y chromosomes of *S. latifolia* are more similar to copies in the respective chromosome than to copies in the other sex chromosome or the autosomes. Moreover, LTR sequences coming from the Y chromosome are less variable than the X-linked or autosomal LTRs. This phenomenon can be explained by the mechanism of gene conversion.

2. Materials and methods

2.1. Microdissection of chromosomes, PCR amplification of LTRs and DNA sequence analysis

Mitotic X and Y sex chromosomes and autosomes were microdissected and collected as described by Hobza et al. (2004). Ten individual Y and X chromosomes, or 110 autosomes (10 of each), were used for each PCR reaction. To amplify *Retand* LTRs we used PCR with the primers: LTR-F1 (5′-TTCCGGGTGTA-ATTCCAGAG-3′) and LTR-R1 (5′-CATATTCTGCACCCGCT-GAC-3′), that produced dominant PCR product with a length of about 600 bp (Kejnovsky et al., 2006). The reactions were performed in a volume of 50 μl, and the final concentration of reagents was: 0.2 mM dNTP, 0.2 μM primers, 1× buffer (TopBio) containing 1.5 mM $MgCl_2$, and 0.6 U of *Taq* polymerase (TopBio). Initial denaturation was followed by 25 cycles of 50 s at 94 °C, 1 min at 55 °C, and 1 min at 72 °C, with a final extension for 7 min. The PTC-200 thermal cycler (MJ Research) was used. PCR products were purified using the Qiagen PCR purification kit, cloned using the pGEM-T Easy cloning system (Invitrogen), and sequenced using the BigDye Terminator sequencing kit and an ABI Prism 3130xl-DNA sequencer (Applied Biosystems). All sequences were read twice and the clone sequences were assembled with Staden Package software (Staden, 1996). The GenBank accession numbers for all the sequences analyzed in this paper are: DQ683758–DQ683969 and DQ922567–DQ922629. Multiple sequence alignments were performed with ClustalW (Thompson-Stewart et al., 1994) and phylogenetic trees were constructed with PHYML (Guindon and Gascuel, 2003; Guindon et al., 2005) using the following parameters: HKY model, estimated frequency of invariants, estimated transition over transversion ratio, gamma-distribution: 4 site categories, alpha parameter estimated.

2.2. Fluorescence in situ hybridization

In order to synchronize germinating seeds of *S. latifolia* (seeds come from the seed collection of the Institute of Biophysics, Brno, Czech Republic), the DNA polymerase inhibitor aphidicolin was added for 12 h, and mitoses were then accumulated with oryzalin. Slides were treated as described in Lengerova et al. (2004) with slight modifications. Slide denaturation was performed in 7:3 (v/v) formamide:2× SSC for 2 min at 72 °C. Slides were immediately dehydrated through 50%, 70%, and 100% ethanol (−20 °C), and air dried. The probe was denatured at 70 °C for 10 min, and 100 ng of the denatured probe was added and hybridized for 18 h at 37 °C. Slides were analyzed using an Olympus Provis microscope, and image analysis was performed using ISIS software (Metasystems). DNA was labeled with Fluorolink Cy3-dUTP (Amersham Pharmacia Biotech) in combination with the nick translation mix (Roche).

3. Results and discussion

3.1. Amplification of Retand long terminal repeats (LTRs) on microdissected chromosomes and their phylogenetic analysis

We amplified LTRs from microdissected Y chromosomes, X chromosomes and autosomes by primers designed according to the *Retand-1* element (Kejnovsky et al., 2006). Amplification resulted in a single PCR product which was cloned to construct three separate libraries. We sequenced 73 Y chromosome-derived LTRs, 86 X chromosome-derived LTRs, and 116 LTRs of autosomal origin.

The sequences of all LTRs were aligned with ClustalW and the phylogenetic tree was constructed using PHYML (Guindon and Gascuel, 2003; Guindon et al., 2005). The phylogenetic analysis showed that LTRs from the Y chromosome as well as those from the X chromosome form separate groups divergent from the LTRs amplified on the autosomes. The Y-linked LTRs had only several larger groups (Fig. 1, the larger Y group comprises ~50 LTRs). Moreover, the Y-linked LTRs had a very high within group similarity that was not regularly found for the X-linked and only rare for the autosomal LTRs. Table 1 reports the redundancy among LTRs, the number of polymorphic sites and the mean pair-wise differences between LTR sequences, and LTR nucleotide diversity. Genetic diversity was highest in the sample of LTR sequences from the autosomes, and lowest in LTRs from the Y chromosome, and pair-wise differences

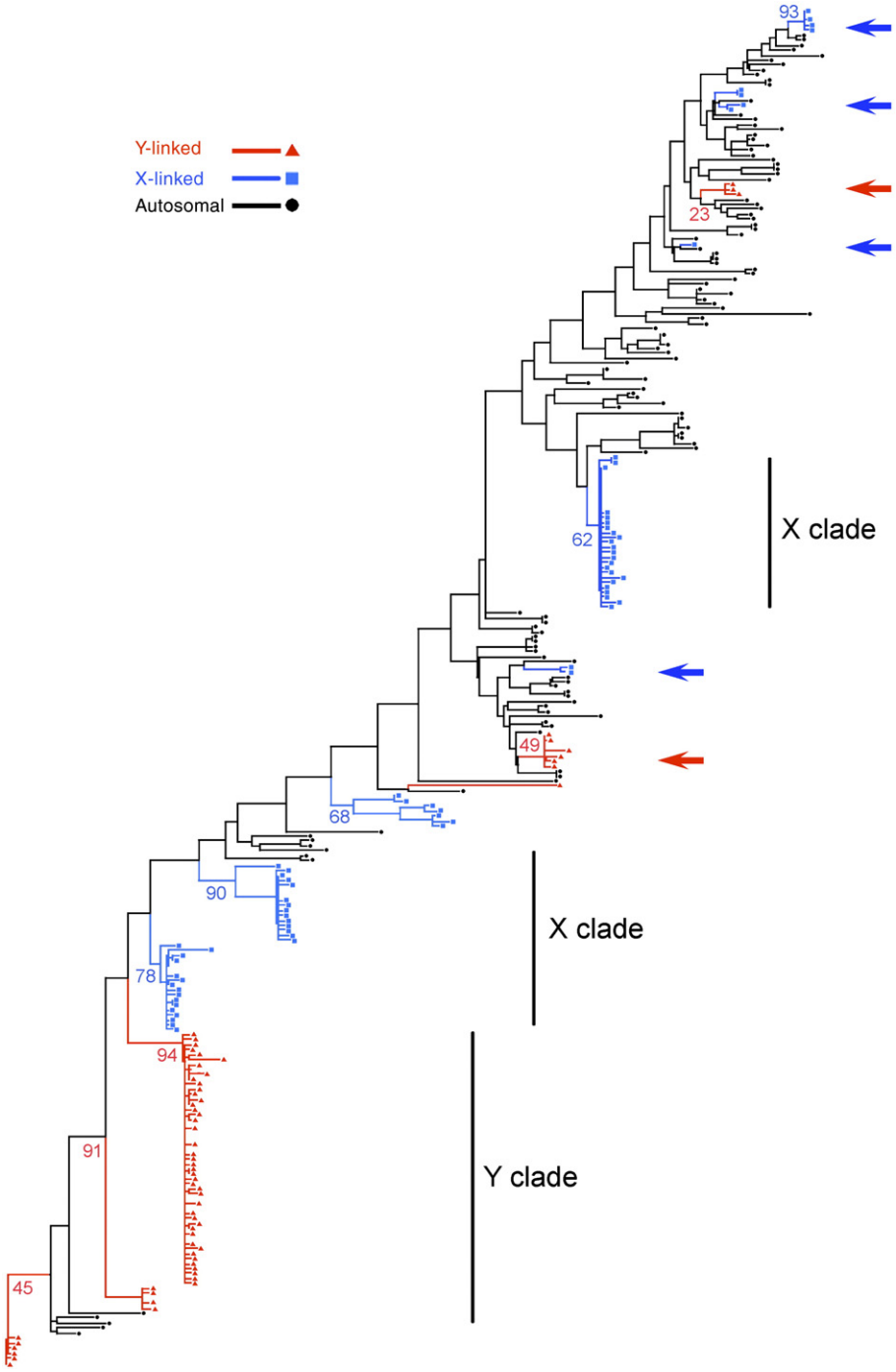

Fig. 1. Phylogenetic analysis of LTRs amplified on the X and Y chromosomes and autosomes of *S. latifolia*. LTR sequences were aligned with ClustalW (Thompson-Stewart et al., 1994), the phylogenetic tree was constructed using PHYML software (Guindon and Gascuel, 2003; Guindon et al., 2005). The tree is unrooted. Several sequences which were truncated were not involved in tree construction but are presented in Table 1. In this tree, the labels of the sequences correspond to the chromosome-of-origin — X chromosomes (blue squares), Y chromosomes (red triangles) or autosomes (black circles). Red or blue arrows indicate Y or X chromosome-derived LTRs of autosomal type, respectively. Black solid lines on the right side mark X- or Y-clades. Non-parametric bootstraping with 500 replicates was done for this tree. Only the values for the X- and Y-clades (>2 sequences) are indicated.

between LTR sequences were lowest for Y-derived LTRs, which reflects the strong tendency for these sequences to form groups in the phylogenetic analysis.

An interesting phenomenon is the presence of several Y or X chromosome-derived LTR sequences among autosome-derived LTR sequences (see the arrows in Fig. 1). We suggest that these LTR sequences of the autosomal type located on the X or Y chromosomes could be a result of their recent transfer from autosomes to the sex chromosomes (more frequently than vice versa). Similarly, the *SlAP3* MADS box gene has been

Table 1
Molecular diversity of LTR sequences derived from *S. latifolia* chromosomes

LTRs derived from	# LTRs sequenced	# redundant LTRs (%)[a]	# polymorphic sites	Mean pair-wise differences (±sd)	Nucleotide diversity (±sd)
X chromosome	86	26 (30%)	159	33.72 (14.84)	0.09 (0.04)
Y chromosome	73	16 (22%)	149	22.57 (10.05)	0.06 (0.03)
Autosomes	116	15 (10%)	277	37.45 (16.41)	0.10 (0.05)

[a] Redundant LTR sequences determined after removal of all gaps.

duplicatively transferred from an autosome onto the Y chromosome in *S. latifolia* (Matsunaga et al., 2003). Gene traffic on and off a sex chromosome, mostly by retrotransposition, has been already reported for the human X chromosome (Emerson et al., 2004; Khil et al., 2005).

3.2. Chromosomal localization of Retand LTRs by FISH

In order to know the relative representation of LTRs of *Retand* elements on different chromosomes we studied chromosomal localization of the *Retand* elements by fluorescence *in situ* hybridization (FISH). When the LTR was used as a probe for FISH on metaphase chromosomes of *S. latifolia*, strong subtelomeric signals were present on all chromosomes (Fig. 2). The only one exception was the Y chromosome where signal was absent on the p arm (Lengerova et al., 2003, 2004). From this we can derive that the Y chromosome harbors only about half the number of *Retand* LTRs present on the other chromosomes. In addition to subtelomeric signals, dispersed signals covering also other parts of chromosomes were observed. In most chromosomes both subtelomeres of the same chromosome carried a signal of similar intensity, only a few had rather asymmetric signal intensity.

3.3. Gene conversion, preferential intrachromosomal retrotransposition or methodological caveat?

A higher intrachromosomal similarity seen in Fig. 1 and a low level of nucleotide polymorphism among copies presented in Table 1 of the Y- or X-linked LTRs could be explained by homogenization of the elements on the sex chromosomes by processes such as gene conversion. This process is, according to our data, more intensive on the Y chromosome, where the homogeneity of LTRs is much higher. Alternatively, the low variability of Y-derived LTR sequences could be a consequence of loss of diversity within local populations due to genetic drift. Filatov (2000) and Ironside and Filatov (2005) described the lower variability in Y-linked genes to their X-linked homologues in *S. latifolia* and discussed the role of selective sweep, background selection and Muller's ratchet. These processes are predicted to severely affect the evolution of the non-recombining Y chromosome and could also account here described phenomena. However, all studies comparing differences between rates of mutation and/or fixation among sex-linked *vs.* autosomal genes were based on comparison of orthologous or allelic sequences. Here we are comparing paralogous sequences — all the sequences belong to the *Retand* family of the same individual.

Our results are interesting in the light of the recent discoveries in mammals where gene conversion was proposed to explain a higher similarity than expected between some X–Y gene copies (Pecon Slattery et al., 2000; Marais and Galtier, 2003; Skaletsky et al., 2003). Gene conversion stands also behind the high identity of large palindromes on the human Y chromosome (Skaletsky et al., 2003; Rozen et al., 2003). This process seems to protect essential genes from degeneration that is an inevitable consequence of lack of meiotic recombination (Charlesworth, 2003; Charlesworth and Charlesworth, 2000). The lack of a meiotic pairing partner probably leaves the Y chromosome free to fold back onto itself (Hurtles and Jobling, 2003). Based on our data, we suggest that this mechanism of intrachromosomal gene conversion on the Y chromosome is even more efficient that gene conversion working on the other chromosomes undergoing normal meiotic pairing. We can speculate that homogenization of *Retand* LTRs could be somehow connected with its occurrence at subtelomeres where tandem repeats, which could be a good target for gene conversion, are located in *S. latifolia* (Buzek et al., 1997).

Chromosome-specific pattern of *Retand* LTRs can be alternatively explained by preferential intrachromosomal retrotransposition, i.e., new copies of retrotransposons can integrate into the chromosome-of-origin resulting in the observed similarities within chromosomes. This is, however, unlikely because transcripts of retrotransposons leave the nucleus for reverse transcription prior to integration. Some targeting of

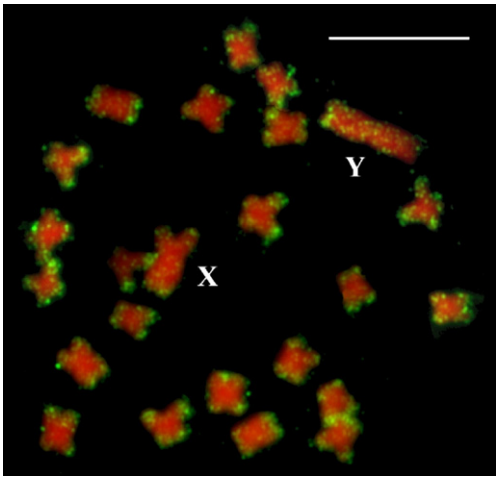

Fig. 2. Metaphase chromosomes of *S. latifolia* were hybridized with LTR. Chromosomes were counterstained with DAPI (here in red); probes were labeled with Cy3-conjugated nucleotides (here signals are in green). The X and Y chromosomes are indicated, bar = 10 μm.

elements was demonstrated for particular chromosomal regions, such as telomeric or subtelomeric repeats as well as rDNA loci (Anzai et al., 2001; Xie et al., 2001; Zhu et al., 2003). However, the chromosomal specificity of integration necessary to explain our results is not known. Higher homogeneity of Y-linked LTRs could be also explained by many more recent retrotranspositions of active *Retand* elements directed onto the Y chromosome in contrast to older X chromosomal or autosomal *Retand* insertions. Thus, recently inserted elements should be more homogenous than more divergent older elements.

The three different libraries of the LTRs that we have made (the X, Y, autosomes) do not have the same complexity (i.e., number of non-redundant clones). Assuming a similar number of *Retand* copies at each subtelomere, we expect the autosomal library to have a greater complexity because it contains LTRs from 11 different chromosomes. The Y library should have the lowest complexity because it contains LTRs from the only Y chromosome for which the *Retand* copies are located only at the asymmetric subtelomere. Finally, the X library should have a slightly higher complexity than the Y because it has *Retand* at both subtelomeric regions. With 73 sequenced clones from the Y library, 86 sequenced clones from the X library, and 116 from the autosomal one, we have differences in sampling effort between libraries. The chance to sequence several times the same copy is quite strong in the case of the Y chromosome, a bit lower for the X, and it is very low for the autosomes. Repeated sequencing of the same copies could explain the existence of groups of homogeneous sequences for the X and Y chromosomes (Fig. 1). However, the sequences belonging to the Y or X groups are not identical as expected in the case of repeated sequencing of the same copies, which tend to suggest that the methodological caveat mentioned can not explain our data. In addition, we designed the test for ascertainment bias. We re-sampled X and Y sequences to get the same number of sampled sequences per chromosome between sex chromosomes and autosomes, we consider that we had 116 *Retand* sequences from 11 autosomes, which gives ~10 *Retand* sequences per autosome, so we re-sampled 10 of 86 *Retand* X sequences and 10 of 73 *Retand* Y sequences. In all 5 re-samplings the phylogenetic trees were similar to the original tree containing all sequences, i.e. LTRs from the Y chromosome as well as those from the X chromosome formed separate groups divergent from the LTRs amplified on the autosomes (data not shown).

Our results support the idea of a high intensity of homogenization on the Y chromosome in *S. latifolia*. However, in another dioecious plant *Rumex acetosa* possessing two Y chromosomes (XY_1Y_2), the variability of Y-associated satellite DNAs was shown to be higher than variability of autosomal satellites (Navajas-Perez et al., 2005). This result supports a contradictory hypothesis of lower rates of sequence evolution and homogenization of the two non-recombining Y chromosomes. Since the Y chromosomes of *R. acetosa* are evolutionary older than the Y chromosome of *S. latifolia*, the differences in the intensity of homogenization could reflect the different stages of the Y chromosome evolution. If the repetitive elements are more diverged, as could be true for the older Y chromosome, then gene conversion is likely not to work on them.

3.4. Conclusions

In summary, we conclude that:

(1) There is a higher intrachromosomal than interchromosomal similarity of LTRs of *Retand* retrotransposons amplified from microdissected sex chromosomes of *S. latifolia*,
(2) Intrachromosomal similarity of *Retand* LTRs originating from the Y chromosome is higher than in the case of X chromosome.
(3) We suggest that gene conversion homogenizing LTRs is a more probable explanation of their higher intrachromosomal similarity than preferential intrachromosomal retrotransposition.

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References

Anzai, T., Takahashi, H., Fujiwara, H., 2001. Elimination of active Tad elements during the sexual phase of the *Neurospora crassa* life cycle. Fungal Genet. Biol. 33, 49–57.

Buzek, J., et al., 1997. Isolation and charecterization of X chromosome-derived DNA sequences from a dioecious plant *Melandrium album*. Chromosome Res. 5, 57–65.

Charlesworth, B., 2003. The organization and evolution of the human Y chromosome. Genome Biol. 4 (Art. No. 226).

Charlesworth, B., Charlesworth, D., 2000. The degeneration of Y chromosomes. Philos. Trans. R. Soc. Lond., B Biol. Sci. 355, 1563–1572.

Elder Jr., J.F., Turner, B.J., 1995. Concerted evolution of repetitive DNA sequences in eukaryotes. Q. Rev. Biol. 70, 297–320.

Emerson, J.J., Kaessmann, H., Betran, E., Long, M., 2004. Extensive gene traffic on the mammalian X chromosome. Science 303, 537–540.

Filatov, D.A., 2000. Low variability in a Y-linked plant gene and its implications for Y-chromosome evolution. Nature 404, 388–390.

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.

Guindon, S., Lethiec, F., Duroux, P., Gascuel, O., 2005. PHYML Online—a web server for fast maximum likelihood-based phylogenetic inference. Nucleic Acids Res. 33, 557–559.

Hobza, R., Lengerova, M., Cernohorska, H., Rubes, J., Vyskot, B., 2004. FAST-FISH with laser beam microdissected DOP-PCR probe distinguishes the sex chromosomes of *Silene latifolia*. Chromosome Res. 12, 245–250.

Hood, M.E., Katawczik, M., Giraud, T., 2005. Repeat-induced point mutation and the population structure of transposable elements in *Microbotryum violaceum*. Genetics 170, 1081–1089.

Hurtles, M.E., Jobling, M.A., 2003. A singular chromosome. Nat. Genet. 34, 246–247.

Ironside, J.E., Filatov, D.A., 2005. Extreme population structure and high interspecific divergence of the *Silene* Y chromosome. Genetics 171, 705–713.

Kass, D.H., Batzer, M.A., Deininger, P.L., 1995. Gene conversion as a secondary mechanism in SINE evolution. Mol. Cell. Biol. 15, 19–25.

Kejnovsky, E., Kubat, Z., Macas, J., Hobza, R., Vyskot, B., 2006. *RETAND*: a novel family of gypsy-like retrotransposon harboring an amplified tandem repeat. Mol. Gen. Genom. 276, 254–263.

Khil, P.P., Oliver, B., Camerini-Otero, R.D., 2005. X for intersection: retrotransposition both on and off the X chromosome is more frequent. Trends Genet. 21, 3–7.

Krzywinski, J., Sangare, D., Besansky, N.J., 2005. Satellite DNA from the Y chromosome of the malaria vector *Anopheles gambiae*. Genetics 169, 185–196.

Le, Q.H., Wright, S., Yu, Z., Bureau, T., 2000. Transposon diversity in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. U. S. A. 97, 7376–7381.

Lengerova, M., Moore, R.C., Grant, S.R., Vyskot, B., 2003. The sex chromosomes of *Silene latifolia* revisited and revised. Genetics 165, 935–938.

Lengerova, M., Kejnovsky, E., Hobza, R., Macas, J., Grant, S.R., Vyskot, B., 2004. Multicolour FISH mapping of the dioecious model plant, *Silene latifolia*. Theor. Appl. Genet. 108, 1193–1199.

Liao, D., 2003. Concerted evolution. In: Encyclopedia of the Human Genome. Macmillian Publishers Ltd, Nature Publishing Group, London, pp. 1–6.

Mantovani, B., 1998. Satellite sequence turnover in parthenogenetic systems: the apomictic triploid hybrid *Bacillus lynceorum* (*Insecta*, *Phasmatodea*). Mol. Biol. Evol. 15, 1288–1297.

Marais, G., Galtier, N., 2003. Sex chromosomes: how X–Y recombination stops. Curr. Biol. 13, R641–R643.

Maside, X., Bartolome, C., Charlesworth, B., 2003. Inferences on the evolutionary histrory of the S-element family of *Drosophila melanogaster*. Mol. Biol. Evol. 20, 1183–1187.

Matsunaga, S., Isono, E., Kejnovsky, E., Vyskot, B., Kawano, S., Charlesworth, D., 2003. Duplicative transfer of MADS box gene to a plant Y chromosome. Mol. Biol. Evol. 20, 1062–1069.

Navajas-Perez, R., et al., 2005. Reduced rates of sequence evolution of Y-linked satellite DNA in *Rumex* (*Polygonaceae*). J. Mol. Evol. 60, 391–399.

Okumura, K., Kiyama, R., Oishi, M., 1987. Sequence analysis of extrachromosomal *Sau*3A and related family DNA: analysis of recombination in the excision event. Nucleic Acids Res. 15, 7477–7489.

Pecon Slattery, J., Sanner-Wachter, L., O'Brien, S.J., 2000. Novel gene conversion between X–Y homologues located in the nonrecombining region of the Y chromosome in *Felidae* (*Mammalia*). Proc. Natl. Acad. Sci. U. S. A. 97, 5307–5312.

Rozen, S., et al., 2003. Abundant gene conversion between arms of palindromes in human and ape Y chromosome. Nature 423, 873–876.

Roy, A.M., et al., 2000. Potential gene conversion and source genes for recently integrated *Alu* elements. Genome Res. 10, 1485–1495.

Schlotterer, C., Tautz, D., 1994. Chromosomal homogeneity of *Drosophila* ribosomal DNA arrays suggests intrachromosomal exchanges drive concerted evolution. Curr. Biol. 4, 777–783.

Schueler, M.G., Higgins, A.W., Rudd, M.K., Gustashaw, K., Willard, H.F., 2001. Genomic and genetic definition of a functional human centromere. Science 294, 109–115.

Skaletsky, H., et al., 2003. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature 423, 825–837.

Smith, G.P., 1976. Evolution of repeated DNA sequences by unequal crossover. Science 191, 528–535.

Staden, R., 1996. The Staden sequence analysis package. Mol. Biotechnol. 5, 233–241.

Thompson-Stewart, D., Karpen, G.H., Spradling, A.C., 1994. A transposable element can drive the concerted evolution of tandemly repetitious DNA. Proc. Natl. Acad. Sci. U. S. A. 91, 9042–9046.

Warburton, P.E., Willard, H.F., 1995. Interhomolog sequence variation of alpha satellite DNA from human chromosome 17: evidence for concerted evolution along haplotypic lineages. J. Mol. Evol. 41, 1006–1015.

Xie, W.W., Gai, X., Zhu, Y., Zappulla, D.C., Sternglanz, R., Voytas, D., 2001. Targeting of the yeast Ty5 retrotransposon to silent chromatin is mediated by interactions between integrase and Sir4p. Mol. Cell. Biol. 21, 6606–6614.

Zhu, Y., Dai, J., Fuerst, P.G., Voytas, D.F., 2003. Controlling integration specificity of a yeast retrotransposon. Proc. Natl. Acad. Sci. U. S. A. 100, 5891–5895.