Genetica 100: 197-204, 1997.

The chromosomal distributions of Ty1-*copia* group retrotransposable elements in higher plants and their implications for genome evolution

J.S. (Pat) Heslop-Harrison', Andrea Brandes[1], Shin Taketa[2], Thomas Schmidt[1,3], Alexander V. Vershinin[1], Elena G. Alkhimova[1,4], Anette Kamm[1,3], Robert L. Doudrick[5], Trude Schwarzacher[1], Andreas Katsiotis[6], Sybille Kubis[7,1], Amar Kumar[8], Steven R. Pearce[8,9], Andrew J. Flavell[9] & Gill E. Harrison'

[1] *John Innes Centre Norwich NR4 7UH, UK;* [2] *Research Institute for Bioresources Okayama University Chuo 2-20-1, Kurashiki Okayama 710, Japan;* [3] *Present address: Institute of Crop Science and Plant Breeding University of Kiel Olshausenstrasse 40 D 24118 Kiel, Germany;* [4] *Laboratory of Cell Population Genetics Institute of Molecular Biology National Academy of Sciences of Ukraine Zabolotnogo St 170 Kiev, Ukraine;* [5] *USDA Forest Service Southern Institute of Forest Genetics Harrison Experimental Forest, 23332 Highway 67 Saucier, Mississippi 39574, USA;* [6] *Present address: Lab. of Molecular Biology Agricultural University of Athens Iera Odos 75 118 55 Athens, Greece;* [7] *Norman Borlaug Institute De Montfort University Scraptoft Campus Leicester LE7 9SU, UK;* [8] *Scottish Crop Research Institute Invergowerie Dundee DD2 5DA, Scotland;* [9] *Medical Sciences Institute The University Dundee DD1 4HN, Scotland*

Accepted 22 April 1997

Key words: in situ hybridization, centromeres, retrotransposons, genome organization, evolution, sequence evolution, *Arabidopsis*, barley

Abstract

Retrotransposons make up a major fraction – sometimes more than 40% – of all plant genomes investigated so far. We have isolated the reverse transcriptase domains of the *Tyl-copia* group elements from several species, ranging in genome size from some 100 Mbp to 23 000 Mbp, and determined the distribution patterns of these retrotransposons on metaphase chromosomes and within interphase nuclei by DNA:DNA *in situ* hybridization. With some exceptions, the reverse transcriptase domains were distributed over the length of the chromosomes. Exclusion from rDNA sites and some centromeres (e.g., slash pine, 23 000 Mbp, or barley, 5500 Mbp) is frequent, whereas many species exclude retrotransposons from other sites of heterochromatin (e.g., intercalary and centromeric sites in broad bean). In contrast, in the plant *Arabidopsis thaliana*, widely used for plant molecular genetic studies because of its small genome (c. 100 Mbp), the *Tyl-copia* group reverse transcriptase gene domains are concentrated in the centromeric regions, collocalizing with the 180 bp satellite sequence pAL1. Unlike the pAL1 sequence, however, the Ty1-*copia* signal is also detectable as weaker, diffuse hybridization along the lengths of the chromosomes. Possible mechanisms for evolution of the contrasting distributions are discussed. Understanding the physical distribution of retrotransposons and comparisons of the distribution between species is critical to understanding their evolution and the significance for generation of the new patterns of variability and in speciation.

Introduction

Genetic maps made using single- or low-copy DNA sequences and molecular markers are now available for many species. The markers and maps are proving valuable for marker-assisted selection in plant breeding programmes, for targeted gene cloning, and for assisting fundamental investigations of plant genome structure (Schwarzacher, 1994). However, there is an important requirement to understand the comparatively large scale organization of the plant genome, including the arrangement of not only single copy but also of repetitive DNA sequences.

Repetitive sequences, consisting of nucleotide motifs ranging from 2 to 10 000 or more base pairs repeated hundreds up to many thousands of times in the genome represent a major fraction of the genome in all plant species: from 40% in species with small genomes, such as those in the rose and horse-chestnut tree genera and *Arabidopsis thaliana*, to 90% or more in species such as wheat or pine with genomes many hundreds of times larger (Flavell, 1986), but containing similar numbers of genes. The variation in genome size in plants contrasts with some birds: genome size was found to vary less than two-fold among a sample of 13.5 bird species representing 17 diverse taxonomic orders (Tiersch & Wachtel, 1991). Whether this indicates a fundamental difference in genome structure between kingdoms – like the lack of polyploidy among most animals – is unknown, but potentially of great significance.

Plant repetitive DNA sequences include retrotransposons, the subject of this review manuscript, and tandemly repeated sequences, as well as dispersed or semi-dispersed motifs not known to amplify through retrotransposition. It is now well known that retrotransposons of the Tyl-copia group are ubiquitous in plants (Flavell, 1992; Flavell, Pearce & Kumar, 1994; Kumar, 1996; see Kumar et al., Flavell et al., this volume) and constitute a significant fraction of all plant genomes. Forty percent or more of the total genome may consist of retrotransposon sequences, based on estimates using the reverse transcriptase domain of *Tyl-copia* as a probe in broad (or field) bean *(Vicia faba*; Pearce et al., 1996a; 1996b). Extensive genomic sequencing showed 50% of extended genomic regions were derived from retrotransposons in maize (Bennetzen et al., 1994; Bennetzen et al., 1996; San Miguel et al., 1996), and even single families of retroelements represented a major fraction of the barley genome (Manninen & Schulman, 1993; Vershinin et al., this volume).

In the present work, we describe the chromosomal distribution of retrotransposons. We present data from *in situ* hybridization of *Tyl-copia* group retrotransposable elements to chromosomal preparations of various species of plants, including angiosperms and gynmosperms, and discuss this in relation to the evolution and diversification of plant genomes and mechanisms of amplification of the elements.

Materials and methods

We used either cloned retrotransposable elements or the PCR product from the reverse transcriptase gene of the Ty1-*copia* locus for *in situ* hybridization. In brief, synthetic degenerate oligonucleotide primers encoding the amino acid sequences for two conserved regions of the reverse transcriptase gene were used for PCR amplification of Tyl-copia regions from genomic DNA of the species under investigation using techniques described previously (Flavell et al., 1992). The PCR fragments were labelled by re-amplification in the presence of modified nucleotides (digoxigenin-11-dUTP or biotin-16-dUTP) and used for *in situ* hybridization. In other cases, clones of highly repetitive sequences from recombinant DNA libraries of genomic DNA were selected and sequenced. Those confirmed as including fragments of *Tyl-copia* retroelements were labelled using PCR, nick-translation, or random-primed labelling. Other probes were obtained from the sources cited and labelled using similar methods.

Chromosome preparations were made from root-tips or buds/apical meristems using standard spreading techniques (Schwarzacher, Leitch & Heslop-Harrison, 1994) with various modifications to optimize the number of metaphases obtained and the yield of well-spread chromosomes free of cytoplasm from each species. Briefly, tissue was fixed in 3:1 ethanol : acetic acid, digested with a pectinase : cellulase enzyme mixture, squashed on a glass slide in 45% acetic acid, and air dried after removal of the coverslip. Preparation of extended DNA fibres followed methods described by Brandes et al. (1997b).

Fluorescent *in situ* hybridization used methods described by Heslop-Harrison et al. (1991). Briefly, the labelled probe in a dextran sulphate, salt-sodium citrate (SSC), salmon sperm DNA, and formamide mixture was denatured, applied to the slide, covered with a coverslip and the preparation and mixture denatured. Hybridization between probe and target DNA on the chromosomes was then allowed to occur overnight at 37 °C before washing in SSC-formamide mixtures. The most stringent wash allowed sequences of more than approximately 85% homology between probe and target to remain stably hybridized. Sites of hybridization of probe to chromosomes were detected with anti-digoxigenin-FITC antibodies (fluorescing green) or Cy3-conjugated streptavidin (red), and chromosomes were counterstained with DAPI (blue fluorescence).

Results and discussion

The general pattern of the genomic distribution of Ty1-*copia* group retrotransposable elements revealed by *in situ* hybridization shows the elements are distributed throughout most of the length of plant chromosomes, with a few regions of higher and lower relative concentration in many species (Pearce et al., 1996a, 1997; Brandes et al., 1997a). Figure la, b shows the results of *in situ* hybridization to metaphase chromosomes from barley (2n = 14) of a cloned fragment (885 bp) of a Tyl-copia group retrotransposon from the same species. Dispersed hybridization of the probe along the chromosome arms is detected. Superimposed upon this pattern are regions with concentrations and depletions of the elements with respect to the distribution along chromosomal arms, in particular near the centromeres and telomeres. We observed no obvious differences between the hybridization pattern using the heterogeneous reverse transcriptase domain and the cloned probe (cf., Figure lb with Figure 1 in Kumar et al., this volume, showing use of the reverse transcriptase domain as a probe; see also Waugh et al., 1997).

Figure 2 shows a double target *in situ* hybridization to metaphase chromosomes of *Arabidopsis thaliana* ($2n = 10$). Because of its small genome size and rapid life cycle, this plant is extensively used as a model for studies of plant molecular genetics, and, along with rice, is likely to be one of the first plant genomes to be sequenced. The sequence pAtMR1 (Murata, Ogura & Motoyoshi, 1994; homologous to the pAL1 family, Martinez-Zapater, Estelle & Somerville, 1986) is present at the centromeres of all five chromosome pairs (Maluszynska & Heslop-Harrison, 1991) and strong hybridization is seen. The reverse transcriptase gene domain of the *Tyl-copia* group elements hybridizes strongly in this region. *Tyl-copia* also gives weak hybridization along the chromosome arms. In Figure 3, DNA fibres from *Arabidopsis* nuclei have been spread and hybridized with the same two sequences. One fibre shows only the presence of the pAtMR1 sequence, whereas the second (lower) shows regions of homology to both probes, demonstrating the physical interspersion of the two sequences. Figure 4 shows a contrasting distribution pattern in onion: the *Tyl-copia* group elements are concentrated at the ends of the prophase chromosomes, with some dispersion along the whole chromosome length (Pearce et al., 1996b).

The dispersal pattern seen throughout the length of the chromosomes in many species is consistent with our knowledge of the mode of replication and insertion of *Tyl-copia* elements in plants. In all plant species so far examined by *in situ* hybridization with LTR retrotransposons, the elements are found, at least at the cytological level, throughout the genome, with a few regions of amplification or depletion (see below). In contrast, many other repetitive DNA sequences, such as satellite sequences or ribosomal DNA sequences, show clustering at a small number of genomic regions (Figures 1, 2). Although only so far investigated in barley (Pedersen & Linde-Laursen, 1994), sugar beet (Schmidt & Heslop-Harrison, 1996) and *Arabidopsis* (Brandes et al., 1997b), many microsatellite sequences used as probes to chromosomes show clustering at particular chromosomal regions with some dispersed signals.

In the plant genomes, some regions of depletion or amplification of retroelements are observed. Exclusion from rDNA sites and some centromeres is frequent (see Figure 1 for barley, or Kamm et al., 1996 for slash pine, *Pinus elliottii*, and Schmidt, Kubis & Heslop-Harrison, 1995, for sugar beet). Many species exclude the domains from other sites of heterochromatin (e.g., intercalary and centromeric sites in broad bean; Pearce et al., 1996a), while onion (Figure 4; Pearce et al., 1996b) and *Arabidopsis* (Figures 2, 3; Brandes et al., 1997a) show amplification in regions where tandemly repeated DNA sequences are present.

At least seven interrelated causes may give rise to the relative differences in occurrence of retrotransposons in different genomic regions, leading to the uneven distribution patterns seen (Figures 1, 2, 4). Depletion in certain regions may arise because:

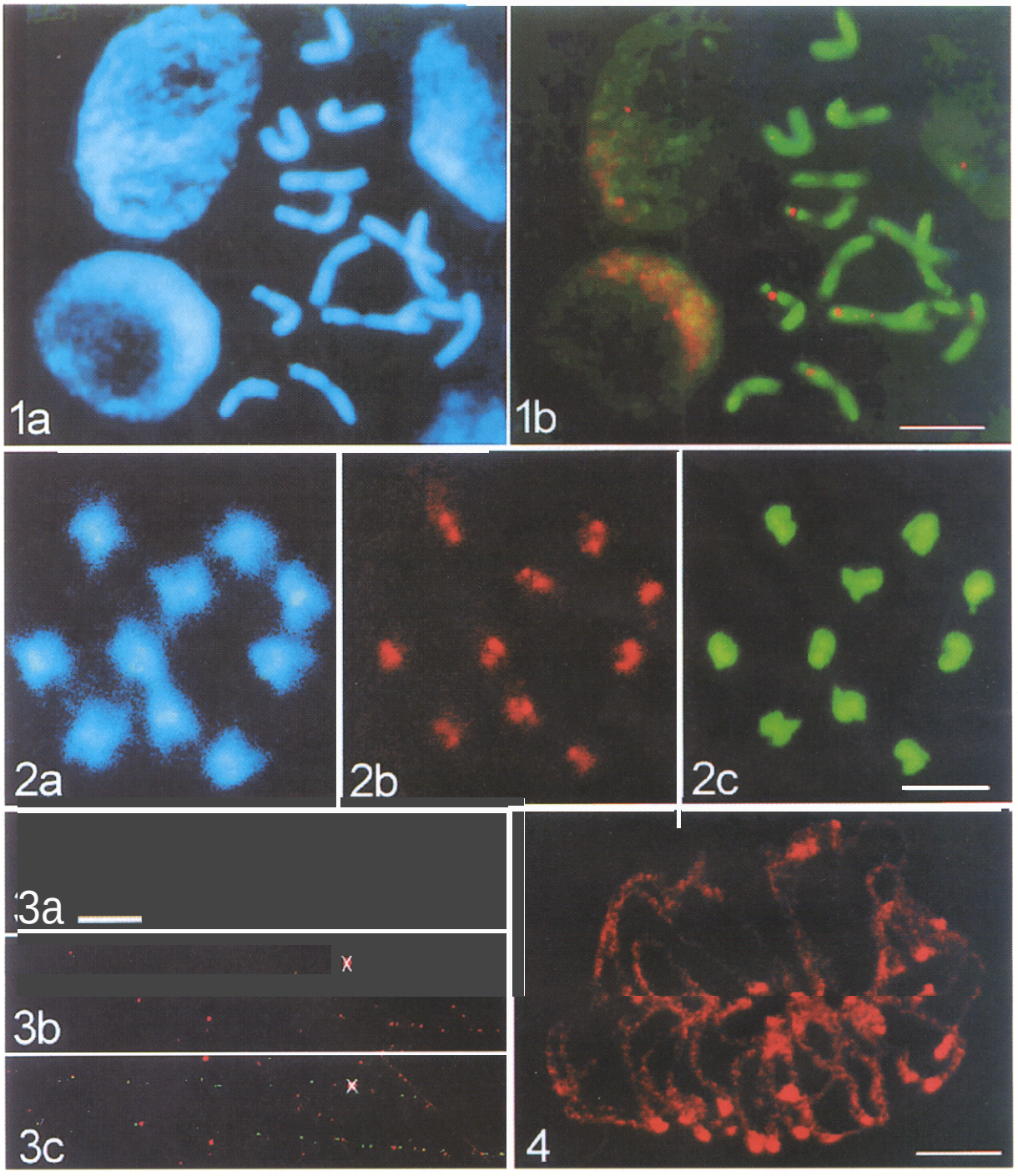
(1) Insertion (in the regions where depletion is seen) gives selective disadvantage, is lethal, or causes sterility.

(2) Insertion target sites are not present in certain regions, or DNA is in a conformation where insertion is not possible.

(3) Some regions of the genome show rapid sequence homogenization and an evolution rate that is much higher than that of retroelements, so any copies that are inserted are lost (which also implies that retroelements are not amplified along with these other sequences); or

(4) Retroelements are selectively deleted after insertion into particular chromosomal regions.

Regions with increased relative concentration of retroelements may be detected because of the above reasons and absolute increases in concentration may occur because:



Figures 1–4. (1) *In situ* hybridization of a cloned sequence of a 885 bp fragment of a Ty1-*copia* group retrotransposon to metaphase chromosomes and interphase nuclei of barley (*Hordeum vulgare*, $2n = 14$). (a) DAPI staining showing chromosomes and interphase nuclei. (b) Double target *in situ* hybridization showing the dispersed distribution of the retrotransponsons, with exclusion from centromeric regions and major clusters of rDNA (labelled red by *in situ* hybridization); (2) Metaphase chromosomes of *Arabidopsis thaliana* ($2n = 10$) stained with DAPI (a) and (b) following *in situ* hybridization with the conserved domain of the reverse transcriptase gene amplified by PCR from genomic DNA (red). (c) Localization of the major paracentromeric sequence, pAtMR1 at the centromeres of all 10 chromosomes. The retrotransposon is mostly collocated with the centromeric sequence, but also, unlike that sequence, shows some dispersed copies along all chromosome arms; (3) *In situ* hybridization to extended DNA fibres from *Arabidopsis* nuclei showing high-resolution localization of (a) the major tandemly repeated satellite sequence pAtMR1/pAL1 (green, cf., Figure 2b) and (b) the reverse transcriptase domain of Ty1-*copia* group retroelements. (c) shows the two images after overlaying. The location of each sequence is visualized as a line of points of hybridization; two lines are seen from the satellite, while the lower line also shows the retrotransposon sequence (left hand end of fibre); (4) Hybridization of the reverse transcriptase domain of Ty1-*copia* retrotransposons to a prophase nucleus of *Allium cepa*, onion. The elements are concentrated at the ends of the chromosomes in the regions of terminal heterochromatin with some dispersion along the whole chromosome length.

(5) Selective insertion of retroelements in some regions occurs preferentially to others.
(6) Amplification of retroelements in some regions might occur not through reverse transcription and re-insertion but through replicative mechanisms (also involved with other non-retrotransposable repetitive sequences) such as unequal crossing over, replication slippage, or perhaps even transposition through DNA intermediates and preferential insertion into linked sites; or
(7) Retrotransposons are ancient genomic components (perhaps facilitating or even causing the change from unstable but autocatalytic RNA to the DNA-based nucleus with transcription into RNA) and they play a key role in the genome, amplifying and homogenizing during evolution.

There is evidence that many of these mechanisms act together at least under some circumstances during genome evolution (see below). Where regions of DNA exclude retrotransposons, there are frequently large blocks of tandem repeats – at the centromeres and major rRNA gene loci, as in barley (see Figure 1), or in sub-telomeric regions as in rye (Pearce et al., 1997). It is possible that the tandemly repeated sequences are packaged tightly or do not have suitable sequence motifs so the integrase enzymes are unable to function in these regions. Alternatively, mechanisms of genome homogenization may act rapidly and retroelements are lost during this process. It is clear that the rDNA sequences may homogenize rapidly in many species (Wendel, Schnabel & Seelaman, 1995), making this an important mechanism for retroelement exclusion for these loci. In the genus sugar beet, it is notable that retrotransposons are excluded (Schmidt, Kubis & Heslop-Harrison, 1995) from the evolutionarily recent sequence present around the centromere (found only in one section of the genus), whereas the ancient tandemly repeated sequences, present throughout the whole genus at intercalary positions, do not exclude retrotransposons (Schmidt & Heslop-Harrison, 1994, and Schmidt, personal communication).

In contrast to the depletion discussed above, in *Arabidopsis* the retroelements tend to be clustered in the centromeric DNA (Figures 2, 3). One can speculate that insertion in many regions of the single copy DNA along the arms (which include little repetitive DNA) disrupts genes or control systems. Hence, even if retrotransposon insertion occurs, many of these plants will die or be sub-fertile, and the retroelements mostly accumulate in genomic regions more tolerant to insertion, such as the centromeric DNA. It is clear from the fibre *in situ* hybridization experiment (Figure 3; Brandes et al., 1997) that the retroelements and centromeric repetitive sequences, pAtMR1, are interspersed. Long stretches of dots are observed, indicating the continuous presence of pAL1 or pAtMR1 repeat units. The reverse transcriptase gene unit is observed in other regions of the same DNA strand, as shown by the pAtMR1 hybridization; as only some 260 bp of the 5 kb Tyl-copia sequence is used as a probe, gaps may represent segments of the retrotransposon not homologous to the repeat unit or other sequences present within the reverse transcriptase gene, including retroelements and other unrelated repetitive DNA sequences (Brandes et al., 1997b; Thompson et al., 1996). The DNA fibre *in situ* hybridization method has great potential to give high resolution data about the organization of the retrotransposons without resorting to large scale genomic sequencing. Using multi-target *in situ* hybridization, it will be possible to locate the different regions of the retroelements and show their insertion and re-insertion patterns in the genome. The result showing interspersion of the centromere repeats and retrotransposons supports results from extensive sequencing of large clones from *Arabidopsis* (Pélissier et al., 1996).

Retrotransposon turnover is likely to occur, with certain families becoming amplified and others becoming lost. Although direct evidence for loss of whole families is not available, amplification without associated loss of retroelements would increase genomic complexity and lead to a general evolutionary increase in genome size. The evolutionary processes that lead to loss of retroelements are poorly understood, and we cannot say whether they are specific to retrotransposons in their action. Equally, retrotransposons may amplify by the same mechanisms as other DNA sequences including both genes and tandemly repeated sequences – by unequal crossing-over, duplication, or via transposition without an RNA intermediate. The relative contribution of the DNA replicative mode amplification and the RNA mode is unclear. Increasing amounts of sequence information combined with knowledge about mutation rates in the two modes of evolution and about the structure of large genomic regions should enable separation of the two evolutionary modes.

All plants within the taxonomic tribe of major temperate cereals such as wheat, barley, and rye, the Triticeae, and related tribes such as the oat tribe have genome sizes in the range of 5000 to 9000 Mbp per haploid chromosome set. The classes of retroelements present in each genome are relatively diverged

from those that were presumably present in the common ancestor (Katsiotis, Schmidt & Heslop-Harrison, 1997), although it is unlikely that the ancestor had a smaller genome than the extant species (Bennett, Smith & Heslop-Harrison, 1982). In the hexaploid oat crop, Katsiotis, Schmidt and Heslop-Harrison (1996) have shown that either individual retroelements or pooled DNA probes isolated from the diploid ancestors, hybridize largely to the chromosomes of the genome-of-origin of the retrotransposon. Thus the retrotransposons are diverged enough that they become essentially genome (species) specific, and they have not homogenized in the hexaploid crop (consistent with the lack of activity analysed by Pearce et al., 1997). In other plant families, one can make a case that genome size or complexity has increased with evolutionary distance, although the contribution of Ty1-*copia* group retroelements to this size increase is unknown. Perhaps ***Arabidopsis*** represents an extant primitive karyotype and genome in the cabbage family Crucifereae, and other genera, such as the cabbage genus ***Brassica*** (where ***B. nigra, B. oleracea,*** and ***B. campestris*** have $n = 8, 9$ and 10 respectively) represent hexaploids with reduced chromosome numbers (Parkin et al., 1995; Lagercrantz & Lydiate, 1995; Sharpe et al., 1995). These ***Brassica*** species also have considerably larger genomes, even considered per 'haploid' chromosome set (c. 100 Mbp for ***Arabidopsis*** vs. 800-900 Mbp for the ***Brassica*** species). In the bean genus, *Vicia*, there is no clear correlation between copy number of Ty1-***copia*** group retroelements and genome size (Pearce et al., 1996a). The ancient fern species, morphologically little changed since the Caboniferous era, might be interesting to examine in more detail. Hybridization of the PCR product probe to the fern ***Pteris,*** often grown in houses, provides evidence for two classes of chromosomes, perhaps a result of polyploidy (Brandes et al., 1997a).

The timing and rate of amplification of retroelements is of great significance to their role in speciation, generation of diversity and genome evolution. Tissue culture of tobacco and rice protoplasts has been shown to lead to activation of elements, as detected by northern blotting (Pouteau et al., 1991; Hirochika et al., 1996). In rice, extensive analysis has shown that the increase in the genomic copy number, detected by southern hybridization, of some families of elements occurs over a 12 to 36 month period (Hirochika et al., 1996). It has been suggested that activation of elements may occur in the widespread and successful opportunistic asexually reproducing (apomictic) dandelion (***Taraxacum*** *officinale*; Richards, 1989). It is also possible that some of the effects described as 'somaclonal variation' in species as diverse as oil palm and potato may be due to activation and insertion of retroelements into genes.

In our studies, we were unable to detect activity of retrotransposons by northern blotting in sugar beet (***Beta*** *vulgaris*; Schmidt, Kubis & Heslop-Harrison, 1995), but a high amount of transcript of the BARE-l retrotransposon family was found in rye, barley and wheat but not oat (Pearce, Kumar & Flavell, 1996c). Very low levels of activity are difficult to detect unequivocally by reverse-transcriptase PCR (RT-PCR) methods because of contamination of RNA with the ubiquitous elements and other reasons (see Pearce et al., 1996, for discussion of the avoidance of this problem using repeated rounds of DNase treatment and poly-A selection). In a wider context, it is known that stress on a plant can activate retrotransposons (Wessler, 1996), thus giving a new range of variability of high significance to colonization of marginal (and presumably stressful) habitats, as discussed by McClintock (see 1984) with respect to transposons. Indeed, the promoters of retrotransposon expression are wound- and stress-inducible (Mhiri et al., 1997). The genomic divergence following retrotransposon activity may lead to speciation because meiotic pairing would be disturbed in hybrids if the insertions occurred in regions of the genome critical for chromosome alignment.

With respect to target site preferences for retrotransposon insertion, there is evidence that at least some sub-families show preferential insertion in some genomic regions. In maize, the elements tend to insert into preexisting retroelements and intergenic spacers (San Miguel et al., 1996). In contrast, Hirochika et al. (1996) sequenced regions surrounding the insertion sites of eight newly-amplified elements of a Ty1-***copia*** sub-family in rice and found only one in repetitive DNA, although the rice genome is about 70% repetitive. Four were inserted in known coding gene sequences, while three were in single-copy but as yet uncharacterized DNA. These data indicate that different retrotransposon sub-families found in any species may behave differently with respect to control of both their amplification and insertion, presumably features of variation in the genes within each retroelement family.

Why plant genomes should vary so much, and what the significance is for plant development and evolution, are important questions (Bennett, Smith & Heslop-Harrison, 1982; Bennett & Leitch, 1995), the answers

to which may shed light on the processes of speciation and generation of diversity. Study of modes of expansion and contraction of the karyotype are a related and significant research topic (D'Amato, 1991; Rees, 1986; Rees & Jones, 1972; Seal, 1983). Knowledge of this area is essential for studies ranging from evolutionary biology through gene expression, for understanding chromosome behaviour and meiosis, to facilitating gene transfer by transformation or sexual methods, and in plant breeding. Examination of the changes that have occurred during long periods of plant evolution indicate the types of change that plant breeders will be able to make in plant genomes during selection.

In conclusion, understanding the genomic and physical distribution of retrotransposons is critical to understanding their evolution and significance. Comparisons of the distribution between species show substantial differences, indicating the variation in plant genome structure. Retroelements play an important role in the generation of the new patterns of variability and in speciation. As a major component of all plant genomes studied, it is vital to learn more about the evolution and distribution mechanisms of Ty1-*copia* group retrotransponsons.

Acknowledgements

We thank British Biotechnology and Biological Sciences Research Council for grants to AJF, A. Kumar, JSHH, and T. Schwarzacher, the Commission of the European Community for grants under Framework IV Human Capital and Mobility to A. Katsiotis and T. Schmidt, and Biotechnology-Biodiversity initiative to JSHH, A. Kumar and AJF, the US Department of Agriculture-Forest Service for a grant to JSHH and A. Kamm, FAZIT for support of T. Schmidt, the Royal Society for a grant to EGA and INTAS for a cooperative research grant to JSHH and AVV.

References

Bennett, M.D. & I.J. Leitch, 1995. Nuclear DNA amounts in angiosperms. Ann. Bot. 76: 113-176.

Bennett, M.D., J.B. Smith & J.S. Heslop-Harrison, 1982. Nuclear DNA amounts in angiosperms. Proc. R. Soc. Lond. B 216: 179-199.

Bennetzen, J.L., K. Schrick, P.S. Springer, W.E. Brown & P. SanMiguel, 1994. Active maize genes are unmodified and flanked by diverse classes of modified, highly repetitive DNA. Genome 37: 565-576.

Bennetzen, J.L., P. SanMiguel, C-N. Liu, M. Chen, A. Tikhonov, A.C. Oliveira, Y-K. Jin & Z. Avramova, 1996, Microcollinearity and segmental duplication in the evolution of grass nuclear genomes, pp. l-3 in Unifying Plant Genomes. 50th SEB Symposium, edited by Heslop-Harrison, Company of Biologists, Cambridge.

Brandes, A., J.S. Heslop-Harrison, A. Kamm, S. Kubis, R.L. Doudrick & T. Schmidt, 1997a. Comparative analysis of the chromosomal and genomic organization of Tyl-copiu-like retrotransposons in pteridophytes, gymnosperms and angiosperms. Plant Mol. Biol. 33: 11-21.

Brandes, A., H. Thompson, C. Dean & J.S. Heslop-Harrison, 1997b. Multiple repetitive DNA sequences in the paracentromeric regions of *Arabidopsis thaliana* L. Chromosome Res. 5: 238-246.

D'Amato, F., 1991. Nuclear changes in cultured plant cells. Caryologia 44: 217-224.

Flavell, A.J., 1992. Tyl-*copia* group retrotransposons and the evolution of retroelements in the eukaryotes. Genetica 86: 203-214.

Flavell, A.J., E. Dunbar, R. Anderson, S.R. Pearce, R. Hartley & A. Kumar, 1992. Ty1-*copia* group retrotransposons are ubiquitous and heterogeneous in higher plants. Nucl. Acids Res. 20: 3639-3644.

Flavell, A.J., S.R. Pearce & A. Kumar, 1994. Plant transposable elements and the genome. Curr. Op. Genet. Devel. 4: 838-844.

Flavell, R.B., 1986. Repetitive DNA and chromosome evolution in plants. Phil. Trans. R. Soc. Lond. B 312: 227-242.

Heslop-Harrison, J.S., T. Schwarzacher, K. Anamthawat-Jónsson, A.R. Leitch, M. Shi & I.J. Leitch, 1991. In *situ* hybridization with automated chromosome denaturation. Technique 3: 109-115.

Hirochika, H., K. Sugimoto, Y. Otsuki, H. Tsugawa & M. Kanda, 1996. Retrotransposons of rice involved in mutations induced by tissue culture. Proc Natl Acad Sci USA 93: 7783-7788.

Kamm, A., R.L. Doudrick, J.S. Heslop-Harrison & T. Schmidt, 1996. The genomic and physical organization of Ty1-*copia*-like sequences as a component of large genomes in *Pinus elliottii* var. *elliottii* and other gymnosperms. Proc. Natl. Acad. Sci. USA 93: 2708–2713.

Katsiotis, A., T. Schmidt & J.S. Heslop-Harrison, 1997. Chromosomal and genomic organization of Ty1-*copia*-like retrotransposon sequences in the genus *Avena*. Genome 39: 410–417.

Kumar, A., 1996. The adventures of the Ty1-*copia* group of retrotransposons in plants. Trends Genet. 12: 41–43.

Lagercrantz, U. & D.J. Lydiate, 1995. RFLP mapping in *Brassica nigra* indicates differing recombination rates in male and female meiosis. Genome 38: 255-264.

Maluszynska, J. & J.S. Heslop-Harrison, 1991. Localization of tandemly repeated DNA sequences in *Arabidopsis thaliana*. Plant J. 1: 159-166.

Manninen, I. & A.H. Schulman, 1993. BARE-l, a *copia*-like retroelement in barley *(Hordeum vulgare* L.). Plant Mol. Biol. 22: 829-846.

Martinez-Zapater, J.M., M.A. Estelle & C.R. Somerville, 1986. A highly repeated DNA sequence in *Arabidopsis thulium*. Mol. Gen. Genet. 204: 417–423.

McClintock, B., 1984. The significance of responses of the genome to challenge. Science 226: 792-801.

Mhiri, C., J-B. More, S. Vernhettes, J.M. Casacuberta, H. Lucas & M-A. Grandbastien, 1997. The promoter of the tobacco Tntl retrotransposon is induced by wounding and by abiotic stress. Plant Mol. Biol. 33: l-10.

Murata, M., Y. Ogura & F. Motoyoshi, 1994. Centromeric repetitive sequences in *Arabidopsis thaliana*. Jap. J. Genet. 69: 361–370.

Parkin, I.A.P., A.G. Sharpe, D.J. Keith & D.J. Lydiate, 1995. Identification of the A and C genomes of amphidiploid *Brassica napus* (oilseed rape). Genome 38: 1122-1131.

Pearce, S.R., G. Harrison, M. Wilkinson, D. Li, J.S. Heslop-Harrison, A.J. Flavell & A. Kumar, 1996a. The Tyl-copia group retrotransposons in *Vicia* species: copy number, sequence heterogeneity and chromosomal localisation. Mol. Gen. Genet. 250: 305–315.

Pearce, S.R., U. Pich, G. Harrison, A.J. Flavell, J.S. Heslop-Harrison, I. Schubert & A. Kumar, 1996b. The Ty1-*copia* group retrotransposons of *Allium cepa* are distributed throughout the chromosomes but are enriched in the telomeric heterochromatin. Chromosome Res. 4: 357-364.

Pearce, S.R., G. Harrison, J.S. Heslop-Harrison, A.J. Flavell & A. Kumar, 1997. Characterisation and genomic organisation of Ty1-*copia* group retrotransposons in rye (*Secale cereale*). Genome, in press.

Pearce, S.R., A. Kumar & A.J. Flavell, 1996c. Retrotransposon expression in potato. Plant Cell Reports 15: 949-953.

Pedersen, C. & I. Linde-Laursen, 1994. Chromosomal locations of four minor rDNA loci and a marker microsatellite sequence in barley. Chromosome Res. 2: 65-71.

Pélissier, T., S. Tutois, S. Tourmente, J.M. Deragon & G. Picard, 1996. DNA regions flanking the major *Arabidopsis thaliana* satellite are principally enriched in Athila retroelement sequences. Genetica 97: 141-151.

Pouteau, S., E. Huttner, M-A. Grandbastien & M. Caboche, 1991. Specific expression of the tobacco *Tnt1* retrotransposon in protoplasts. EMBO J. 10: 1911-1918.

Rees, H., 1986. The consequences of DNA change in chromosomes: *Lathyrus* and lathyrism. Proc. int. symposium sponsored by Inst. de. biocenotique experimentale des agrosystemes (IBEAS), edited by A.K. Kaul and D. Corn. Paris, Third World.

Rees, H. & R.N. Jones, 1972. The origin of the wide species variation in nuclear DNA content. Int. Rev. Cytol. 32: 53-91.

Richards, A.J., 1989. A comparison of within-plant karyological heterogeneity between agamospermous and sexual *Taraxacum* (Compositae) as assessed by the nucleolar organiser chromosome. PI. Syst. Evol. 163: 177-185.

San Miguel, P., A. Tikhonov, Y-K.. Jin, N. Motchoulskaia, D. Zakharov, A. Melake-Berhan, P.S. Springer, K.J. Edwards, M. Lee, Z. Avramova & J.L. Bennetzen, 1996. Nested retrotransposons in the intergenic regions of the maize genome. Science 274: 765-738.

Schmidt, T. & J.S. Heslop-Harrison, 1994. Variability and evolution of highly repeated DNA sequences in the genus *Beta*. Genome 36: 1074-1079.

Schmidt, T. & J.S. Heslop-Harrison, 1996. The physical and genomic organization of microsatellites in sugar beet. Proc. Natl. Acad. Sci. USA 93: 8761-8765.

Schmidt, T., S. Kubis & J.S. Heslop-Harrison, 1995. Analysis and chromosomal localization of retrotransposons in sugar beet *(Beta vulgaris* L.): LINEs and Tyl-copia-like elements as major components of the genome. Chromosome Res. 3: 335-345.

Schwarzacher, T., 1994. Mapping in plants: progress and prospects. Curr. Op. Genet. Devel. 4: 868-874.

Schwarzacher, T., A.R. Leitch & J.S. Heslop-Harrison, 1994, DNA:DNA in *situ* hybridization – methods for light microscopy, pp. 127-155 in Plant Cell Biology: A Practical Approach, edited by N. Harris & K.J. Oparka. Oxford University Press, Oxford.

Seal, A.G., 1983, The distribution and consequences of changes in nuclear DNA content, pp. 225-232 in Kew Chromosome Conference II, edited by P.E. Brandham & M.D. Bennett. George Allen & Unwin, London.

Sharpe, A.G., I.A.P. Parkin, D.J. Keith & D.J. Lydiate, 1995. Frequent nonreciprocal translocations in the amphidiploid genome of oilseed rape (*Brassica napus*). Genome 38: 1112-1121.

Thompson, H., R. Schmidt, A. Brandes, J.S. Heslop-Harrison & C. Dean, 1996. A novel repetitive sequence associated with the centromeric regions of *Arabidopsis thaliana* chromosomes. Mol. Gen. Genet. 253: 247-252.

Tiersch, T.R. & S.S. Wachtel, 1991. On the evolution of genome size of birds. J. Heredity 82: 363-368.

Waugh, R., K. McLean, A.J. Flavell, S.R. Pearce, A. Kumar, B.T. Thomas & W. Powell 1997. Genetic distribution of BARE-l retrotransposable elements in the barley genome revealed by sequence-specific amplification polymorphism (S-SAP). Mol. Gen. Genet. 253: 687-694.

Wendel, J.F., A. Schnabel & T. Seelanan, 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton *(Gossypium)*. Proc Natl Acad Sci 92: 280-284.

Wessler, S.R., 1996. Plant retrotransposons: Turned on by stress. Current Biol. 6: 959-961.