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Ty1-copia group retrotransposons are ubiquitous and heterogeneous in higher plants

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ABSTRACT

We have used the polymerase chain reaction to isolate fragments of *Ty1-copia* group retrotransposons from a wide variety of members of the higher plant kingdom. 56 out of 57 species tested generate an amplified fragment of the size expected for reverse transcriptase fragments of *Ty1-copia* group retrotransposons. Sequence analysis of subclones shows that the PCR fragments display varying degrees of sequence heterogeneity. Sequence heterogeneity therefore seems a general property of *Ty1-copia* group retrotransposons of higher plants, in contrast to the limited diversity seen in retrotransposons of *Saccharomyces cerevisiae* and *Drosophila melanogaster.* Phylogenetic analysis of all these sequences shows, with some significant exceptions, that the degree of sequence divergence in the retrotransposon populations between any pair of species is proportional to the evolutionary distance between those species. This implies that sequence divergence during vertical transmission of *Ty1-copia* group retrotransposons within plant lineages has been a major factor in the evolution of *Ty1-copia* group retrotransposons in higher plants. Additionally, we suggest that horizontal transmission of this transposon group between different species has also played a role in this process.

INTRODUCTION

The retrovirus-related transposon family is one of the major classes of eukaryotic transposon. It includes the vertebrate retroviruses and the LTR retrotransposons of animals, plants and microorganisms (1–5, 20). There are two major groups of LTR retrotransposons, the *Ty1-copia* group and the *gypsy* group, which are distinguished by sequence homology and gene order. LTR retrotransposons are found in a variety of angiosperms (flowering plants; 3,4). One of the reasons for this study was to find out whether this type of transposon is ubiquitous in the angiosperms and whether they are present in the more primitive non-flowering plants.

In particular species, notably the potato, *Ty1-copia* group retrotransposons display a high degree of sequence heterogeneity and are present in high copy number (7). This heterogeneity contrasts with the situation for this type of transposon in *Drosophila melanogaster* and *Saccharomyces cerevisiae*, which contain a small number of homogeneous *Ty1-copia* group elements (5, 8–10). The second reason for this study was to find out whether this intriguing sequence heterogeneity in angiosperms is matched in the other divisions of the higher plant kingdom.

The final reason to look for these sequences in a wide variety of higher plants was to analyse the phylogenetic relationships between the members of this retrotransposon group in the plant kingdom. There has been considerable interest in the evolution of retrotransposons. It has been postulated that there has been horizontal genetic transfer of this class of transposon between different species (11–14). The phylogenetic evidence for horizontal transmission relies upon the isolation of closely related transposon sequences in widely divergent plant species. There are several candidates which support this hypothesis but a more comprehensive study encompassing a wide variety of species whose evolutionary history is well understood would provide not only extra possible examples of horizontal transmission, but also an estimate of the relative impacts of this process and sequence divergence during vertical transmission down evolving lineages, upon the evolution of plant *Ty1-copia* group retrotransposons.

We show here that virtually all higher plant genomes, even the most primitive divisions such as bryophytes, contain *Ty1-copia* group retrotransposons and heterogeneity of these sequences pervades the higher plant kingdom, but its degree is variable. In general, the more closely related plant species contain examples of more closely related *Ty1-copia* group retrotransposons, suggesting that a major factor in the evolution of this group of transposons has been divergence during vertical transmission down evolving plant lineages. Lastly, we find some cases where very distantly related plants carry similar reverse transcriptase sequences, suggesting that horizontal transmission of *Ty1-copia* retrotransposons between plant species has also occasionally occurred.

*X65396–X65443 (incl.); X66797–X66799 (incl.)

MATERIALS AND METHODS

Plant materials

Tissues from the plants in the Table were obtained either from the wild, from the University of Dundee Botanical Garden or from Professor David Cove (*Ceratodon purpureus*). Plant tissues were visually inspected for absence of animal or fungal contamination, then surface-sterilized for 5 min. in 2% hypochlorite, followed by several washes with water prior to plant DNA isolation.

Molecular analysis

DNAs were isolated by the method of Saghai-Marzoof *et al* (6). Primer oligonucleotides were synthesized using an Applied Biosystems 381A synthesizer. PCR reactions were performed on 50ng to 500ng plant DNA using conditions described previously (7). Reaction products were separated on 1.5% agarose gels and visualized under UV illumination. DNA fragments were eluted from gel slices by centrifugation through Costar Spin-X columns and purified by phenol-chloroform extraction followed by ethanol precipitation. The weaker PCR bands were reamplified from dilutions of the eluted DNAs. Prior to cloning, the fragments were treated with T4 polynucleotide kinase followed by Klenow polymerase, to create blunt-ended, 5'-phosphorylated ends which were subcloned into Sma I-digested, phosphatase-treated M13mp18 (7). Nucleotide sequences of single-stranded M13 DNAs were determined by the dideoxy method using Sequenase™ enzyme and the manufacturer's protocol.

Computer analysis

Computing was carried out on the Vax facilities of the University of Leicester and the SERC Computing Centre, Daresbury, UK. Nucleotide sequences were first aligned relative to the corresponding region of the *Tnt1* retrotransposon (15) using the GAP programme of the UWGCG Sequence Analysis Package (16). Frameshifts were corrected by insertion of a nonspecific nucleotide and the resulting peptide sequences were cross-compared using the CLUSTAL sequence analysis programme (17), an updated version of this package (CLUSTAL V) or the PAPA set of programmes (18,19). Any discrepancies between the trees generated by TREE and PAPA3 were resolved by submitting the topology suggested by one program to analysis by the other. In such cases, the tree with the lowest standard deviation (19) was adopted. The alignments within individual plant species were on the DNA sequences and those between species were on peptide sequences. The sequences are deposited in the EMBL database under Accession Numbers X65396–X65443 in the order Pc1-Pc6, Ds1-Ds10, Cp3-Cp11, Pt1-Pt11, Eg1-Eg4, Da1-Da4, Da10-Da11, Gb5-Gb11, Wm2-Wm10 and X66797–X66799 for Da5, Da6 and Da12 (see Figure 1 for the sequences and the species to which they belong).

RESULTS

Multiple *Ty1-copia* group sequences in higher plants

To detect *Ty1-copia* group members in a wide variety of plant genomes by PCR analysis, we used degenerate oligonucleotide primers which can amplify a fragment of the reverse transcriptase gene from far diverged members of this group in plants and animals (7,20). The downstream priming region chosen by us, specifying the peptide sequence YVDDML, is well conserved between *Ty1-copia* group members (12) but is also reasonably conserved among other retroelements. The upstream primer region is much less well conserved and has the advantage that it is completely absent from other retroelements (7). We were therefore reasonably confident that any amplified fragments of the expected size would belong to the *Ty1-copia* retrotransposon group.

To address whether *Ty1-copia* group retrotransposons are virtually ubiquitous in higher plants, DNAs were isolated from a wide variety of species encompassing most of the higher plant divisions (Table 1). All of these DNAs except one generated a band of the expected size upon PCR amplification and other discernible fragments were rarely seen (data not shown). To determine the approximate proportion of these bands which were derived from *Ty1-copia* group retrotransposons, we subcloned 8 fragments from a spectrum of the plant divisions (excluding angiosperms as these had already been covered; 7) and determined the sequences of 70 subclones from these fragments. For each subclone, the nucleotide data were compared with the corresponding region of the Tnt1 retrotransposon of *Nicotiana tabacum* (15). Any frameshifts were corrected and the resulting deduced peptide sequences were compared with *Tnt1*, using the gap alignment program (16). 51 of these subclones were obviously derived from *Ty1-copia* group retrotransposons, as judged by their sequence homologies with *Tnt1* (Figure 1). The sequences of 19 subclones (mainly from *Ginkgo biloba* and *Polytrichum commune*) were unrecognisable (not shown). All 8 species chosen yielded sequences characteristic of *Ty1-copia* group retrotransposons. We conclude from these data, together with previous studies on angiosperm plants (7,14), that *Ty1-copia* group retrotransposons are at least extremely widespread, if not ubiquitous in the higher plant kingdom.

To gain a visual representation of the sequence similarities, we cross-compared the sequences with each other using CLUSTAL (17). The aligned sequences are shown in Figure 1. It was obvious that all of these plants harbour a variety of different *Ty1-copia* group retrotransposon sequences. Therefore, the sequence heterogeneity seen in angiosperm *Ty1-copia* group retrotransposons is not a peculiarity of this division of the higher plant kingdom but is also present in the other divisions.

To get a better idea of the degree of sequence variation among these sequences in non angiosperm plants, we cross-compared each of the subclones from a particular species with the others from the same species, using the TREE and PAPA3 multiple alignment computer programmes (Figure 2; see Materials and Methods). The results in general support the visual comparison, namely all 8 non angiosperm plant species contain a variety of different *Ty1-copia* group retrotransposon sequences.

The sequences in Figure 2 are placed in an order corresponding to the antiquity of the division of the plant kingdom to which they belong. This varies from at least 500 million years for the bryophytes, *Polytrichum commune*, *Dicranum scoparium* and *Ceratodon purpureus* to approximately 370 million years for the gymnosperm spermatophytes *Weltwitschia mirabilis* and *Ginkgo biloba* (21). The degree of sequence heterogeneity shows no correlation with plant division. For example, half of the subclones from the bryophyte *Ceratodon purpureus* are so similar to each other that they are effectively the same transposon sequences (Cp7 and 8 are identical and Cp4 differs by three base pairs in 250). In contrast, none of the sequences from *Dicranum scoparium* (which belongs to the same taxonomic division as *C. purpureus*) are highly similar to each other. The species containing notably

Table 1. Higher Plant Species containing sequences characteristic of *Ty1-copia* group retrotransposons

DIVISION	ORDER	GENUS	SPECIES	COMMENTS
Bryophyta	*Sphagnales*	*Sphagnum*	*capillifolium*	
	Dicranales	*Dicranum*	*scoparium*	Sequence data obtained
	Dicranales	*Ceratodon*	*purpureus*	Sequence data obtained
	Hypnobryales	*Hypnum*	*jutlandicum*	
	Hypnobryales	*Pleurozium*	*sp.*	
	Polytrichales	*Polythrichum*	*commune*	Sequence data obtained
Pteridophyta	*Psilotales*	*Psilotum*	*triquetrum*	Sequence data obtained
	Selaginellales	*Selaginella*	*martensii*	
	Equisetales	*Equisetum*	*giganteum*	Sequence data obtained
	Osmundales	*Osmunda*	*regalis*	No fragment found
	Filicales	*Dicksonia*	*antarctica*	Sequence data obtained
	Marsileales	*Marsilea*	*macropus*	
Spermatophyta -	*Ephedrales*	*Ephedra*	*frustrillata*	
(subdivision,	*Welwitschiales*	*Welwitschia*	*mirabilis*	Sequence data obtained
Gymnosperma)	*Cycadales*	*Cycas*	*revoluta*	
	Ginkgoales	*Ginkgo*	*biloba*	Sequence data obtained
(subdivision, *Angiosperma*	*Magnoliales*	*Magnolia*	*sieboldii*	
class, *Dicotyledoneae*)	*Piperales*	*Piper*	*nigrum*	
	Ranunculales	*Helleborus*	*lividus*	
	Papaverales	*Meconopsis*	*grandis*	
	Cercidiphyllales	*Cercidiphyllum*	*japonicum*	
	Hamamelidales	*Hamamelis*	*virginiana*	
	Urticales	*Pellonia*	*repens*	
	Fagales	*Fagus*	*sylvatica*	
	Betulales	*Betula*	*pendula*	
	Juglandales	*Juglans*	*regia*	
	Caryophyllales	*Saponaria*	*officinalis*	
	Polygonales	*Rheum*	*officinale*	
	Paeoniales	*Paeonia*	*lutea*	
	Passiflorales	*Passiflora*	*quadrangularis*	
	Cactales	*Opuntia*	*ficus-indicus*	
	Violales	*Viola*	*jooi*	
	Begoniales	*Begonia*	*haageana*	
	Salicales	*Populus*	*alba*	
	Primulales	*Primula*	*bulleyana*	
	Thymelaeales	*Daphne*	*mezereum*	
	Saxifragales	*Astilbe*	*thunbergii*	
	Myrtales	*Carica*	*papaya*	
	Rutales	*Citrus*	*grandis*	
	Sapindales	*Aesculus*	*hippocastanum*	
	Geraniales	*Geranium*	*phaeum*	
	Celastrales	*Ilex*	*aquifolium*	
	Rhamnales	*Ceanothus*	*impressus*	
	Oleales	*Ligustrum*	*vulgare*	
	Elaeagnales	*Elaeagnus*	*pungens*	
	Proteales	*Banksia*	*ashbyi*	
	Gentianales	*Gentiana*	*asclepiadea*	
	Asterales	*Senecio*	*huntii*	
(Class, *monocotyledoneae*)	*Liliales*	*Allium*	*cepa bulbiferum*	
	Iridales	*Iris*	*foetidissima-variegatus*	
	Zingiberales	*Musa*	*acuminata*	
	Orchidales	*Maxillaria*	*tenuifolia*	
	Cyperales	*Cyperus*	*papyrus*	
	Commelinales	*Tradescantia*	*cerinthoides*	
	Poales	*Oryza*	*sativa*	
	Arecales	*Elaeis*	*guineensis*	
	Arales	*Pistia*	*stratiotes*	

The orders within each division are ranked according to their degree of relatedness. The Cronquist and Takhtajan taxonomic system (as summarised in reference 22) is used throughout.

heterogeneous sequences are *D. scoparium* and *Psilotrum triquetrum* whereas 50% or more of the subclones in *C. purpureus*, *Equisetum giganteum*, *Dicksonia antarctica*, *G. biloba* and *W. mirabilis* belong to very closely related sequences. *P. commune* is intermediate between these two classes.

Some highly diverged plant species contain closely related *Ty1-copia* group retrotransposons

To look for homologies between the *Ty1-copia* group retrotransposons of different plant species, we submitted the 51 sequences in this study to phylogenetic analysis using the

Figure 1. Alignment of predicted peptide sequences of plant retrotransposon subclones. x = sequence ambiguity; * = nonsense codon, – = gap. The sequence of the corresponding region of *Tnt1* is shown for comparison.

CLUSTAL V multiple alignment program. The phylogenetic tree for all the plant fragments in this study, together with some previously described *Ty1-copia* group retrotransposons of angiosperm plants and animals, is shown in Figure 3. Several conclusions can be drawn from this Figure. First, the more closely related clusters of sequences from Figure 2 remain uninfiltrated by sequences from other species. For example, the Cp4,7,8 and Cp11 sequences, from *C. purpureus*, remain together but other sequences from other plant species, notably the other *Dicranale, D. scoparium*, are more closely related to these than are the other two sequences from this species (Cp3 and Cp10). Second, the more divergent groups of sequences from single species, such as *D. scoparium* (Ds) and *P. triquertrum* (Pt) are spread across the tree, showing that there is as much sequence variation of *Ty1-copia* group retrotransposons within these species as exists within all the species examined. This result is qualitatively similar to that obtained for the angiosperm *Solanum tuberosum* (7). Third, there is some degree of relationship between the sequence divergences and the evolutionary distance separating the host species. For instance, many of the bryophyte-derived sequences (Cp, Ds and Pc) lie within a single branch in the lower half of the tree in Figure 3 and many gymnosperm sequences (Gb and Wm) lie within the branch near the top of the tree. This suggests that these sequences are associated with particular plant divisions and that they have been transmitted vertically down these lineages to the present day.

The final conclusion from Figure 3 is that some highly diverged plant species contain quite closely related *Ty1-copia* group retrotransposons. The best example of this is Cp3 and Gb10, from the bryophyte *C.purpureus* and the gymnosperm *G. biloba*. These two sequences are 67% identical at the predicted amino acid level. Their host species last shared a common evolutionary precursor about 500 million years ago (21). It is highly unlikely that retroelement sequences could retain this relatively high degree of sequence conservation over this period of time (see Discussion) and the most likely explanation for this is that there has been horizontal transmission of this sequence between the two lineages at a more recent point in the past.

DISCUSSION

The *Ty1-copia* group of retrotransposons has been found in a diverse collection of eukaryotes, including fungi, animals and flowering plants. We have shown here that this group of transposons pervades the entire higher plant kingdom. We have also shown that the phenomenon of high sequence heterogeneity, which distinguishes the angiosperm plant *Ty1-copia* group retrotransposons from their fungal and insect counterparts, extends to other divisions of the higher plant kingdom.

The degree of sequence heterogeneity is rather variable among the plant genomes we have examined. There is no correlation with the divisions of the plant kingdom and therefore the source

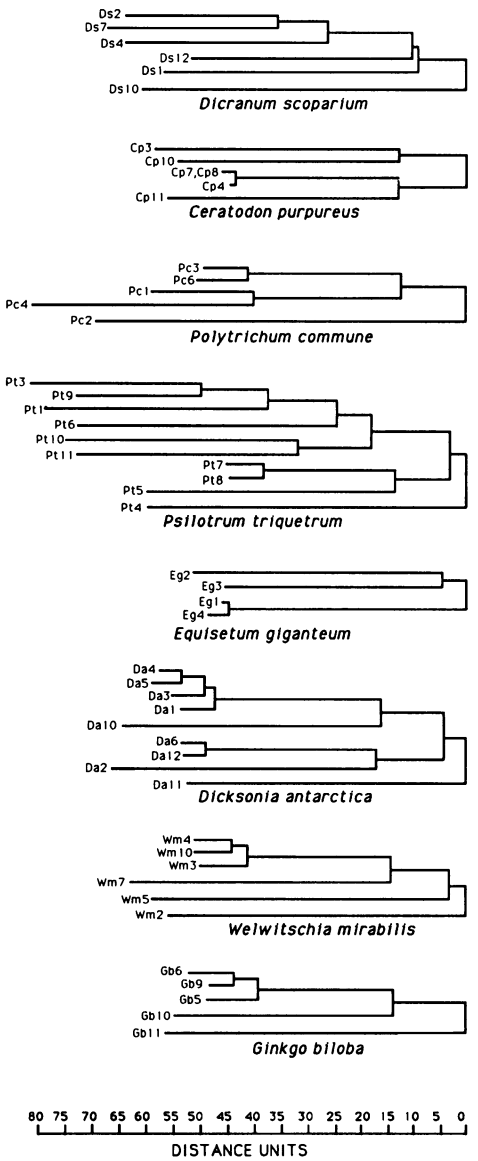

Figure 2. Phylogenetic trees of nucleotide sequences of *Ty1-copia* group members of various higher plant species. Divergences in distance units (18) are indicated by horizontal branch lengths, the vertical lengths have no significance. Because the trees are unrooted, there is no significance to the relative lengths of the two horizontal sections of the rightmost arms joining the most diverged clones (Ds10, Cp3/Cp10, Pc2, Pt4, Eg1/Eg4, Da11, Wm2 and Gb11) to the other sequences. However, the total of the two lengths is equal to the distance separating these from the other sequences.

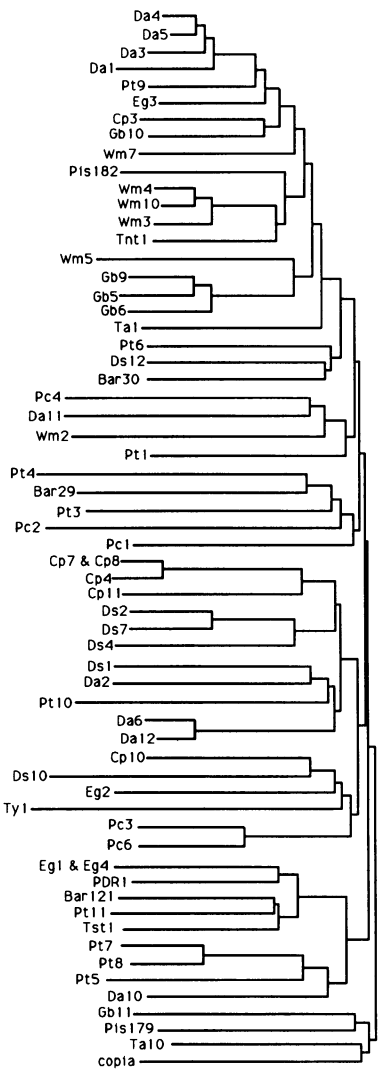

Figure 3. Phylogenetic analysis tree for the sequences in this study and other *Ty1-copia* group sequences. The tree was generated using the CLUSTAL V program. The sequences not from this study are: Pis 179, Pis182, PDR1 (from pea, *Pisum sativum*); Ta1, Ta10 (from *Arabidopsis thaliana*); Tnt1 (from tobacco, *Nicotiana tabacum*); Tst1 (from potato, *Solanum tuberosum*); Bar 29, Bar30, Bar121 (from barley, *Hordeum vulgare*); *copia* (from *Drosophila melanogaster*) and *Ty1* (from *Saccharomyces cerevisiae*). The actual sequences can be found in reference 7).

of this heterogeneity cannot lie in any particular property of any division. One possibility is that the sequence heterogeneity is influenced by the copy number of these transposons (23). The most extreme case of heterogeneity is seen in the potato *Solanum tuberosum* which has hundreds of copies of these transposons (7). *Arabidopsis thaliana*, on the other hand has far fewer *Ty1-copia* group retrotransposons and these are considerably less heterogeneous (14). We have not assessed the copy numbers of the transposons in this study, but the isolation of ten distinct sequences out of ten randomly chosen subclones, in the case of the *P. triquertrum* sequences, suggests that at least this plant species contains many copies of this retrotransposon group.

It is likely that we have not amplified every member of the *Ty1-copia* group retrotransposons in these species. Because these sequences are so heterogeneous, some are likely to diverge so much in the PCR priming regions that they are unamplifiable by our primers. This sets an approximate limit on the elements which we can detect, for instance, *copia*, *1731* and the *Ty* elements are not amplified under our conditions. However, we can amplify extremely divergent sequences (around 70% predicted amino acid divergence).

If copy number influences sequence heterogeneity of the *Ty1-copia* group retrotransposons, it is worthwhile asking what influences the copy number. One plausible contributor may be the genome size of the plants in question. *Solanum tuberosum* has a tetraploid genome of 8×10^9 bp, whereas *A. thaliana* has a diploid genome of 5×10^8 bp. The genome sizes of most of the non-angiosperm plants which we have analysed are not known but *C. purpureus* has a genome of approximately the size of *A. thaliana* (personal communication, Dr Thummler, University of

Munich) and it is interesting that this species displays the least sequence heterogeneity of the plants described here. However, there must be an additional influence on sequence heterogeneity of *Ty1-copia* group retrotransposons over and above that of copy number, because the copy numbers of this group of transposons in the yeast *S. cerevisiae* and the insect *D. melanogaster* are comparable with those in *A. thaliana*, yet the retrotransposons inhabiting the former species are much more homogeneous in their sequences than the *A. thaliana* elements (7–10).

More than one half of the *Ty1-copia* group retrotransposon fragments in this study carry either stop codons or frameshifts in the fragment of the reverse transcriptase reading frame isolated here. Because these subclones comprise about only 6% of the open reading frames of the retrotransposons to which they belong, it is likely that the large majority of the retrotransposons to which these sequences belong are transpositionally defective. This is also the case for this transposon group in *S. tuberosum* and *A.thaliana* (7,14). It therefore seems to be the rule that plant *Ty1-copia* group retrotransposons are transpositionally inactive, though there is at least one notable exception (15). This is perhaps not unsurprising in plants containing large numbers of these elements, considering the deleterious effects of high levels of retrotransposition.

Our phylogenetic analysis suggests that there is a tendency for particular divisions of the higher plant kingdom to carry some broadly similar retrotransposon sequences. This suggests that these sequences were acquired early in the evolution of these divisions and have been transmitted down the germ lines of the diverging species. It thus appears that vertical transmission has played a major role in the evolution of this group of transposons. The general spectrum of heterogeneity within particular plant species is compatible with this hypothesis (7 and this study). During periods of high transpositional activity, some retrotransposon sequences would amplify to produce multi-copy, relatively homogeneous elements which would then diverge, either by subsequent retrotransposition or by natural acquisition of mutations. Thus recent bursts of transposition, superimposed upon more ancient events would create just the kind of heterogeneity spectrum within a species which we see here and have reported previously.

The many examples of closely related species, or even the same species, carrying widely divergent retrotransposon sequences can be rationalised if the extremely high rate of mutation associated with reverse transposition is taken into account. Actively transposing retroelements incorporate mutations at approximately a million-fold the rate of nuclear genes (24). It is therefore not surprising that there are great sequence differences between some LTR retrotransposons from closely related species. What is surprising is the occasional observation of relatively high levels of sequence similarity between *Ty1-copia* group retrotransposons from widely diverged plant species. The most notable example we are aware of is the Cp3-Gb10 pair described here. The nucleotide sequences are 63% identical and the predicted amino acid sequences are 67% identical, yet the two host species are separated by approximately 500 million years of evolution. The nucleotide substitution rate is roughly 10^{-9} per site per year. This is the substitution rate for well conserved cellular genes and about a million-fold lower than the rate for retroviruses (24).

Such a low mutation rate is incompatible with a genetic element which relies upon reverse transcription for its replicative transposition. These elements confer apparently no selectable advantage on their hosts and should therefore evolve at the rate for non coding DNA, which is approximately ten fold faster than that for cellular genes. If they were transmitted vertically with occasional selection for transposition competence, the very act of transposition would accelerate the mutation rate by a factor of 10^5-fold. There is therefore no plausible model based solely upon vertical transmission which can explain this exceptional example of sequence conservation. The only reasonable alternative is that these sequences have been transferred across species barriers much more recently.

Our studies suggest that a high degree of sequence divergence during vertical transmission has been a major influence upon the evolution of *Ty1-copia* group retrotransposons in higher plants but horizontal transmission between species has also played a role. The goals for the future are to find the reasons for this high level of divergence and a mechanism for the horizontal transfers.

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