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Mechanisms and rates of genome expansion and contraction in flowering plants

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Abstract

Plant genomes are exceptional for their great variation in genome size, an outcome derived primarily from their frequent polyploid origins and from the amplification of retrotransposons. Although most studies of plant genome size variation have focused on developmental or physiological effects of nuclear DNA content that might influence plant fitness, more recent studies have begun to investigate possible mechanisms for plant genome expansion and contraction. Analyses of 'relatively neutral' genome components, like transposable elements, have been particularly fruitful, largely due to the enormous growth in genomic sequence information from many different plant species. Current data suggest that unequal recombination can slow the growth in genome size caused by retrotransposon amplification, but that illegitimate recombination and other deletion processes may be primarily responsible for the removal of non-essential DNA from small genome plants.

Introduction

Angiosperm (flowering plant) genomes vary tremendously in nuclear DNA content, including some species with less than 50 megabases (Mb) of DNA per haploid nucleus and others with more than 85,000 Mb (Bennett & Leitch, 1995). Some of this difference is caused by variations in gene number, especially due to recurrent episodes of polyploid formation or segmental duplication, followed by varying degrees of gene loss (Tikhonov et al., 1999; Blanc et al., 2000; Grant et al., 2000; Ku et al., 2000; Vision, Brown & Tanksley, 2000; Wendel, 2000; Bancroft, 2001). However, most of the variation in genome size is caused by differences in the amounts of repetitive DNA (Flavell et al., 1974), especially the class of mobile DNAs known as LTR- (long terminal repeat-) retrotransposons (SanMiguel et al., 1996; Tikhonov et al., 1999; Vicient et al., 1999; Wicker et al., 2001). In maize, LTR-retrotransposons comprise over 60% of the total nuclear DNA (SanMiguel & Bennetzen, 1998; Myers, Tingey & Morgante, 2001). The amplification of LTR-retrotransposons can be a very rapid process, sometimes increasing the copy number of an LTR-retrotransposon several fold in just one plant generation (Hirochika, 1993; Hirochika et al., 1996). In maize, all of the sequenced retrotransposons were shown to have inserted within the last 6 million years, leading to at least a doubling of maize genome size in that time period (SanMiguel et al., 1998).

In 1997, Bennetzen and Kellogg raised the possibility that plant genomes might be headed to an irreversible genomic 'obesity'. This disquieting idea came from the observation that rapid mechanisms (like polyploidy and transposable element amplification) had been identified for genome expansion, but no similarly efficient processes had been identified in plants that could decrease genome size. Phylogenetic data provided some support for a history of genome expansion in flowering plants, but with some lineages that appeared to have undergone genome contraction (Bennetzen & Kellogg, 1997; Kellogg, 1998; Leitch, Chase & Bennett, 1998). However, these analyses were limited by their assumptions that genome size expansion and contraction are equally likely and that the genome size changes that require the fewest steps

are the most likely. In the absence of a comprehensive mechanism for genome size contraction, both of these assumptions may be inappropriate. Hence, as Bennetzen and Kellogg (1997) and Petrov (2001) have pointed out, the search for an efficient mechanism for genome size decrease in flowering plants deserves a high priority. Until such a mechanism is reported, then the seemingly unlikely possibility exists that 'plants may indeed have a one way ticket to larger genome sizes' (Bennetzen & Kellogg, 1997).

Results

Processes that increase genome size

Numerous mechanisms exist for increasing gene number by segmental or full genome duplication. Although de novo gene creation from raw DNA (or RNA) sequence must have occurred early in the history of life on earth, the vast majority of 'new' genes created in the last several hundred million years have been derived from the duplication, rearrangement and divergence of pre-existing genes. This common origin of the wide array of current gene functions is evidenced by the striking homology of most genes between distantly related bacteria and between prokaryotes and eukaryotes.

One simple mechanism for increasing gene number is by the creation of a polyploid. Polyploids arise either by duplication of the genome within a single species (autopolyploidy) or the acquisition of genomes from two closely related species (often via a wide cross) into the same nucleus (allopolyploidy). This process essentially doubles gene number as well as the content of the non-genic DNA within a species (Figure 1). Perhaps all eukaryotes, including even the tiny genome of the yeast *Saccharomyces cerevisiae* (Wolfe & Shields, 1997), have undergone cycles of polyploidization. Polyploids, mostly allopolyploids, are especially frequent among the angiosperms (Wendel, 2000).

Other processes exist that can increase gene number and genome size by segmental duplication, including unequal recombination (Figure 1) and non-reciprocal translocations. However, both of these processes create deletions on one participating chromatid that are exactly equal to the resultant insertions on another chromatid. Hence, only a selective advantage could lead to a net increase in genome size by either of these mechanisms. The loss of essential genes from such a deletion would often provide a strong selection against the genome size decreases generated by these processes, while an increase in gene number might (in a few cases) provide a selection for the gene amplification outcome. Of course, selection is needed in only one of the two directions in order to create a biased outcome. With selection acting in favour of the increased gene number outcome, the DNA between the genes would also be amplified because of its tight linkage to the selected loci.

In the grasses, at least, most genome size increases in the last 10 million years have been caused by the amplification of transposable elements (SanMiguel et al., 1996; 1998; SanMiguel & Bennetzen, 1998; Vicient et al., 1999; Shirasu et al., 2000; Wicker et al., 2001). All transposable elements have the ability to increase their numbers within a genome via transposition (Figure 1). Hence, natural selection should act on these mobile DNAs to increase their copy number so that they become 'selfish' or 'parasitic' (Doolittle & Sapienza, 1980; Orgel & Crick, 1980). Rogue RNA polymerase III transcription products, so called SINEs (small interspersed nuclear elements) like the Alu elements of humans (Boeke, 1997), demonstrate this behaviour unambiguously. In all flowering plants that have been investigated so far, it is the LTR-retrotransposons that are the biggest variable relating to genome size. These elements comprise 60% or more of many large plant genomes like maize, wheat and barley (SanMiguel & Bennetzen, 1998; Vicient et al., 1999; Myers, Tingey & Morgante, 2001; Wicker et al., 2001) but less than 50% of the small rice genome and around 10% of the smaller *Arabidopsis* genome (Deshpande & Ranjekar, 1980; The Arabidopsis Genome Initiative, 2000). The numerous inverted repeat transposable elements, like MITEs (miniature inverted repeat transposable elements) (Wessler, Bureau & White, 1995), are generally too small in size and/or too limited in number to constitute a particularly large percentage of any plant genome.

Processes that can decrease genome size

The loss of whole chromosomes has been observed in unstable polyploids created by the hybridization of distantly related species (Laurie & Bennett, 1989; Riera-Lizarazu, Rines & Phillips, 1996). This outcome should itself be unstable as it alters genic balance, and would only be resolved with the loss (or silencing) of most genes until a tolerated gene ratio was established. Because the intermediates are

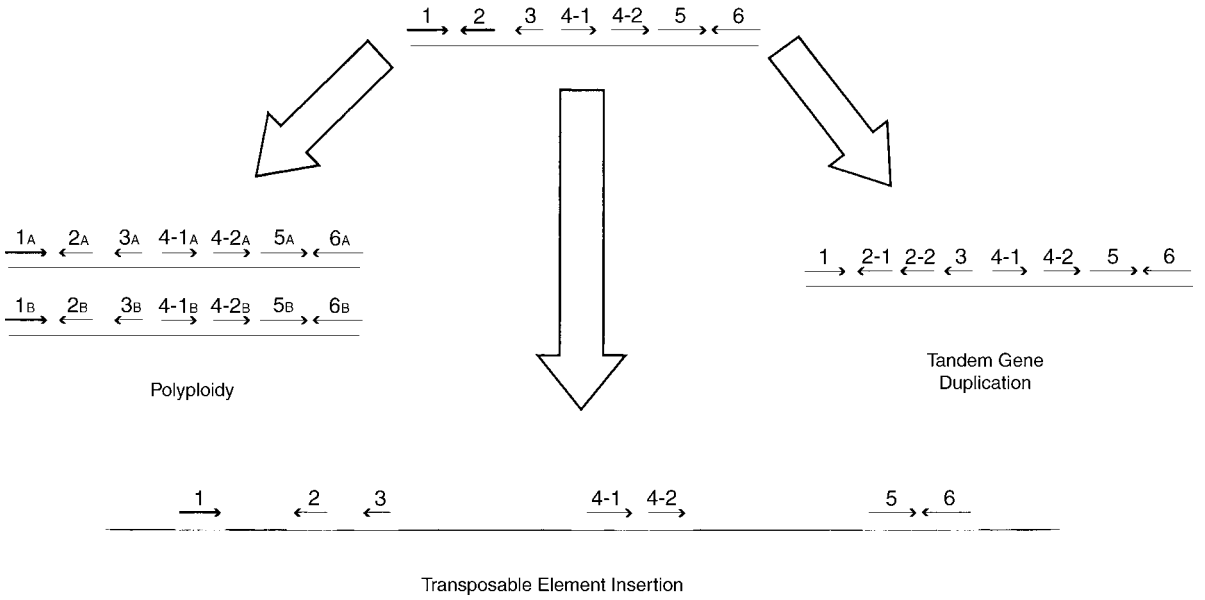

Figure 1. Mechanisms for plant genome expansion. The upper bar shows an initially small genome segment with seven genes indicated by unique numbers and horizontal arrows that depict the location of each gene and its direction of transcription. In the lower left bar, the genomic contribution of this region has doubled through the process of polyploidization. In the central bar, the genome has expanded because of the insertion of numerous transposable elements (dark lines) in the region, including one inside gene 4-1. However, these insertions may not have affected function of any of the genes in the region, including 4-1 if the insertion is in an intron. Plants commonly show similar gene function in colinear segments of different species despite an abundance of gene-flanking repetitive DNAs in one species and their absence in another. In fact, the upper and central lower figures are very similar to the *adh1*-orthologous regions of sorghum and maize (Tikhonov et al., 1999). The lower right figure shows an increase in genome size as an outcome of unequal recombination, in this case involving the duplication of gene 2.

expected to have a low fertility derived from aneuploid gametes, it is not clear how often this process contributes to heritable decreases in genome size in natural populations. The loss of genes from polyploids is observed, sometimes at very high rates, in both recent and ancient polyploids that retain viability (Song et al., 1995; Blanc et al., 2000; Ku et al., 2000; Shaked et al., 2001) but these losses are associated with deletions that are much smaller than whole chromosomes.

Unequal recombination can also decrease genome size but, as mentioned above, one of the two participating chromatids in such an interaction will receive a duplication equal to the deletion on the other participant. Hence, only positive selection for the deletion could lead to a decrease in genome size. When such an unequal recombination event involves a deletion of a gene, for instance from a tandem gene family (Figure 2), it seems more likely that selection (if any) would act against fixation of the deletion rather than in favour of it.

One possible mechanism for unidirectional genome size contraction is unequal intrastrand recombination. In this process, the unequal recombination occurs between two tandem repeats, in direct orientation, that are on the same chromatid. The outcome of such an event is the net deletion of one repeat and the sequences between the repeats on the chromosome and the generation of a DNA circle containing these sequences. The circle is lost, in most cases, thereby creating a net deletion.

In theory, unequal intrastrand recombination could be particularly active in the partial removal of LTR-retrotransposon sequences. Because the long terminal repeats (LTRs) of retrotransposons are in direct orientation and share very high sequence homology at the time of element insertion, they should be excellent substrates for unequal intrastrand recombination. For unequal intrastrand recombination between the LTRs in a single element, the expected deletion outcome is a solo LTR. Solo LTRs have been observed many times in plants (Shepherd et al., 1984; SanMiguel et al., 1996; Chen et al., 1998; Vicient et al., 1999). In barley and its wild relatives, the relative ratio of solo LTRs to intact elements for the *BARE-1* retroelement is inversely proportional to overall genome size, suggesting that this unequal recombination process

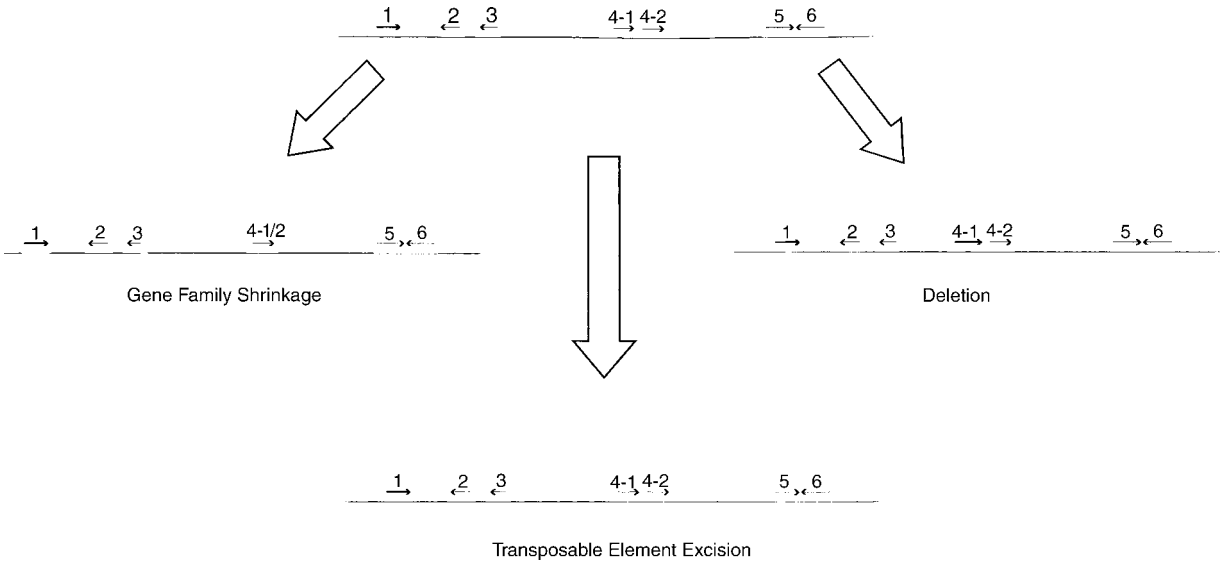

Figure 2. Mechanisms for plant genome contraction. The upper bar shows a segment of DNA from a large genome plant, with dark boxes indicating transposable elements and arrows with numbers indicating the locations and transcriptional directions of genes. The upper bar is identical to the central lower bar in Figure 1. The lower left bar depicts a shrinkage in genome size caused by unequal recombination, in this case between the two copies of gene 4 to create a chimeric single gene. The central lower bar shows a region that has been decreased in size by the transpositional excision of a single transposable element, originally located within gene 4-1. The lower right bar indicates a region that has decreased in size because of the deletion (by an undesignated mechanism) of some of the DNA contained in the retrotransposon repeat block between genes 3 and 4-1.

has contributed to a decrease in overall nuclear DNA content (Vicient et al., 1999).

However, unequal intrastrand chromosomal recombination within LTR-retrotransposons cannot decrease genome size back to the level existing prior to retrotransposon amplification. Amplification of an LTR retrotransposon via transposition, followed by unequal intrastrand recombination to generate a solo LTR, still leaves the genome larger by a single LTR. Hence, this process can attenuate genome size expansion, but cannot reverse it. In their discussion of this issue, Bennetzen and Kellogg (1997) suggested that unequal intrastrand recombination between adjacent LTR retrotransposons of the same family might also occur, leading to an actual net decrease in genome size. Some evidence for such events has now been observed in wheat (Wicker et al., 2001) and maize (P. Miguel, pers. comm.), but its frequency and overall genome size contributions are not known.

Finally, any general preference for deletions relative to insertions in a genome, by whatever mechanism(s), can lead to a progressive decrease in genome size (Petrov, 1997, 2001) (Figure 2). Such a bias toward deletions has been observed in mammals and insects (Graur, Shuali & Li, 1989; Petrov, Lozovskaya & Hartl, 1996). Moreover, a general correlation exists between intron size and genome size in animals (Ogata, Fujibuchi & Kanehisa, 1996), although this difference accounts for only small percentage of overall animal genome size variation (Charlesworth, 1996). This direct correlation between gene and genome size also exists, to a minimal degree, in flowering plants. In one example, Dubcovsky et al. (2001) found that the four genes they analyzed were all smaller in *Arabidopsis* than in either barley or rice. However, the differences (mostly due to intron size variation) were less than 2-fold, and could not explain the more than 30-fold genome size difference between *Arabidopsis* and barley, for instance. Plants tend to have small introns, probably with a fairly small amount of 'neutral space' where mutations can occur without any serious effect on gene function. Hence, a better place to look for genome size variation would be in some numerous, but relatively neutral, portion of the plant nuclear genome.

Petrov and colleagues have set the standard in recent investigation of genome size variation in animals. Investigations of retroelements in insects have shown that deletions are more common than insertions in

these rapidly evolving genome components (Petrov, Lozovskaya & Hartl, 1996). Moreover, Petrov and colleagues demonstrated that at least some insect species with relatively large genomes (Hawaiian crickets of the genus *Laupala*) have a 40-fold lower rate of retroelement sequence loss by deletion than that seen in *Drosophila* (Petrov et al., 2000), indicating that this process could account for a preferential loss of sequences from species that develop small genomes.

Although many truncated or internally deleted versions of plant retroelements have been noted (Jin & Bennetzen, 1989; reviewed in Kumar & Bennetzen, 1999), no comprehensive study has been undertaken to determine the relative frequencies of these events. Of course, if one is searching for factors that might decrease plant genome size, it is logical to search in a plant with a well-characterized and relatively small genome.

Lessons from Arabidopsis

I have recently begun to investigate structural variation in several LTR-retrotransposons of *Arabidopsis thaliana* (unpub. obs.). The existence of a nearly complete genomic sequence of *Arabidopsis* (The Arabidopsis Genome Initiative, 2000) provides a powerful tool for these studies, although any results obtained will be impacted by the lack of completely finished sequence in some of the retrotransposon-rich regions in and around the centromeres.

After inspecting several dozen elements of various different gypsy and copia families of LTR-retrotransposons, I have seen that these elements are much more frequently deleted in *Arabidopsis* than in maize or any other investigated cereal species. Solo LTRs outnumber intact elements. However, numerous elements are truncated by a deletion process that does not appear to involve homologous recombination. Many of these truncated elements have very short sequence homologies (one to a few bp) at the boundaries of the deletion, suggesting that the truncations may have been generated by illegitimate recombination.

Discussion

Genome size variation is expected to be the outcome of dynamic processes for genome size expansion and shrinkage. Numerous studies have uncovered powerful and rapid mechanisms that promote growth in genome size, prominently including polyploidy and LTR-retrotransposon amplification. In plants, molecular mechanisms that can rapidly decrease genome size have not been documented, but frequent deletions associated with the formation of some 'synthetic' allopolyploids suggest that such a mechanism or mechanisms must exist (Song et al., 1995; Shaked et al., 2001). However, processes that give rise to increases or decreases in genome size do not need to be dramatic to be effective, because a slow but steady process can overcome major but rare events (Petrov, 1997, 2001).

A role for natural selection?

Most studies relating to eukaryotic genome size have sought a selective advantage for or against larger genome sizes. Many effects of genome size on the physiology of multicellular eukaryotes have been proposed or demonstrated (Mirsky & Ris, 1951; Sparrow & Mischke, 1961; reviewed in Petrov, 2001). However, these correlation studies are unable to determine whether these variations have actually provided a foundation for selection within a species. As we begin to understand plant genome structure, it seems less and less likely that most individual changes leading to a decreased genome size would often have a selective advantage. If a plant genome of 5000 Mb were to experience the deletion of a 5 kb segment of an LTR-retrotransposon, this would yield a net decrease in genome size of 0.0001%. It is difficult to see how such a difference could have any significant fitness effect, if genome size *per se* were the sole basis for selection (Bennetzen & Kellogg, 1997). If genes were also affected by such a deletion, then (as discussed previously) it is more likely that the deletion would be selected against. Changes in macrosatellite (e.g., maize knob) repeat composition of a genome can occur by segregation of polymorphic variants or by a large deletion, but these repeats are not responsible for most of the variation in genome size seen in plants. The 5–200 kb repeat blocks derived from LTR-retrotransposons (Bennetzen et al., 1994; SanMiguel et al., 1996) appear to be the major variable in plant genome size. Any large deletions (greater than a few hundred kb) of these elements that are intermixed with genes should still be too small to provide any clear selective advantage, but would often be detrimental due to gene loss. Hence, arguments regarding selection for or against genome size are likely to be less fruitful than investigations into a possible unidirectional mechanism for decreases in nuclear DNA content.

Unequal intrastrand recombination

Unequal homologous recombination is a frequent process in all investigated eukaryotes. Apparently, precise regional pairing of chromosomes in meiosis can only minimize but not completely avoid such ectopic recombination. However, unequal recombination between sister chromatids or homologues leads to a reciprocal deletion/duplication on the two participating chromosomes. Without selection, such events might have little net effect on genome size.

Unequal intrastrand recombination, between nearby direct repeats on the same chromosome, can yield a net deletion. This phenomenon presumably occurs in all eukaryotes, although it has not been proven to exist in plants. The presence of this phenomenon in plants is suggested by the abundance of solo LTRs, relative to a general absence of the LTR-internal-LTR-internal-LTR structure that would be the product of an unequal interstrand recombination. However, as discussed above, these unequal events within a single LTR-retrotransposon only remove part of the element, leaving behind a solo LTR. Hence, an intra-element unequal recombination can only slow the rate of genome expansion, not fully reverse it.

Net decreases in genome size can result from unequal intrastrand recombination between two different elements of the same family that are on the same chromatid and in the same orientation. There is some evidence that such events do occur, although their relative frequency appears to be low in *Arabidopsis* (K. Devos & J. Bennetzen, unpub. obs.). However, this might be true only because *Arabidopsis* has relatively few LTR-retrotransposons that are clustered together, at least in the part of the genome that has been sequenced. As Bennetzen and Kellogg (1997) noted, however, recombination (both equal and unequal) in repeated elements outside genes appears to be relatively infrequent and the resultant deletions would often be deleterious if they removed any genes between the flanking transposable elements. Regardless of these caveats, the contribution of unequal intrastrand recombination to genome size variation should be further investigated in plants.

Illegitimate recombination

Several different mechanisms may contribute to the general phenomenon referred to as 'illegitimate recombination'. In general, all of these known or putative mechanisms allow recombination without the involvement of large regions of homology between the participating chromosomal regions. In *E. coli*, illegitimate recombination is several orders of magnitude less frequent than homologous recombination mediated by *recA*. In higher eukaryotes, though, illegitimate recombination may be more.

In plants, double-strand break repair occurs primarily via illegitimate recombination (reviewed in Gorbunova & Levy, 1999). Kirik, Salomon and Puchta (2000) have noted that repair of a double-strand break is more frequently associated with a deletion in *Arabidopsis* than in a larger genome plant, tobacco. Hence, illegitimate recombination might account for the frequent truncation of retrotransposons that is seen in *Arabidopsis* and also for the differences in rates of deletion in different insect species (Petrov et al., 2000).

Prospects

Studies of plant genome size variation will remain productive as long as they focus on the molecular mechanisms responsible for this variation. Routes to increased genome size are now well established, and promising avenues for the study of genome size shrinkage have recently been developed. Investigations of a wide range of species, in a phylogenetically informed manner (Bennetzen & Kellogg, 1997; Kellogg, 1998), will help uncover the reasons for the great variations in genome complexity that now exist. Although micro-alterations in genome size may not provide sufficient material for significant degrees of natural selection, plants must deal with the genome contents that have been generated by mechanisms of shrinkage and expansion. Vastly different genome sizes, possibly arrived at without selection during their incremental progression, will influence how a plant prospers in any given environment. Moreover, any DNA in the nucleus can serve as the raw material for a possible evolved improvement in genetic function. We will only understand how genes operate and evolve in context if we understand the mechanisms and rates of genome size change that determine the nuclear environment.

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