LETTER TO THE EDITOR

Do Plants Have a One-Way Ticket to Genomic Obesity?

Perhaps the most contentious issues discussed at a recent Keystone Symposium ("The Evolution of Plant Development"; held in Taos, NM, in January 1997) were the origin and significance of variations in plant genome size. During these discussions, it became clear that most (perhaps all) of the participants had not grasped many of the central observations that should inform any debate of genome size variation, partly due to the diversity of disciplines (i.e., evolutionary biology, genetics, genomics, and population biology) that contribute to these essential points. Hence, we felt that a short presentation on this subject would be of value to the plant science community.

The fundamental issue is the likelihood of changes in genome size over evolutionary time and, in particular, the likelihood of decreases in genome size versus increases. Although increases—via amplification of retrotransposons—are clearly possible and apparently easy, decreases may be more difficult and/or may occur less frequently. In this letter, we assess the frequency of genome size change, assuming that increases and decreases are equally likely, and contrast that frequency to the one obtained when it is assumed that decreases in genome size are virtually impossible. We then discuss and evaluate some mechanisms that might lead to decreases in genome size.

One of the earliest and most prominent observations on the molecular properties of the genetic material of flowering plants was the tremendous variation in genome size, from the 110 megabase pairs (Mbp) of Arabidopsis to the over 110,000 Mbp of *Fritillaria assyriaca* (reviewed in Bennett and Leitch, 1995). This striking genome size variation between closely related species has been termed the C-value paradox (Thomas, 1971), meaning that it is paradoxical that genomic complexity (i.e., size) does not correlate with the biological complexity of the organism. For instance, although barley and rice differ by about 11-fold in genome size, both plant species appear to have similar morphological complexity and a similar number of biochemical pathways and physiological processes. Moreover, both barley and rice are approximate diploids (with only small segmental duplications) and have roughly the same number of genes (Moore et al., 1995).

Some of this genome-size variation is due to the polyploidy commonly found in the angiosperms or to tandemly repeated satellite sequences (Peacock et al., 1981), but most is associated with ill-defined classes of interspersed highly repetitive and middle repetitive DNAs (Flavell et al., 1974). Recent studies have indicated that the majority of this reiterated DNA is composed of retroelements, several classes of mobile DNA that transpose through an RNA intermediate (Smyth et al., 1989; Moore et al., 1991; Pearce et al., 1996; SanMiguel et al., 1996; Suoniemi et al., 1996). In maize, a particular class of retroelements with long-terminal repeats (LTRs), the retrovirus-like retrotransposons, is most abundant. Retrotransposons comprise at least 50% of the maize nuclear genome and are arranged as complexes of nested elements in the spaces between genes (SanMiguel et al., 1996). Retroelements are found in the genomes of all plant species that have been examined, but they seem to be highly abundant only in species with large genomes. This suggests that retroelements, particularly retrotransposons, account for most of the great variation in plant genome size (SanMiguel et al., 1996).

If retrotransposons are the largest single component of many flowering plant genomes, the question arises as to why all plant genomes have not expanded with the amplification of these elements. Because retroelements all transpose without excision, their mobility will always increase their copy number and thereby increase genome size. Therefore, does continuous or episodic retroelement amplification mean that all plants are on the road to larger genomes, or is there an active process for removing these interspersed repetitive DNAs from plant genomes?

To talk about a possible directionality to genome size from a phylogenetic perspective requires one to make inferences about the genome sizes of ancestral species, and evolutionary biologists have developed a fairly simple logical structure for making such inferences (see, for example, Maddison et al., 1984; Harvey and Pagel, 1991). Briefly, if two closely related species have the same genome size, then it is simplest to assume that their ancestor's genome was also this size. This inference is strengthened if the next most closely-related extant species (the outgroup) also has the same genome size.

If the first two species have different genome sizes, then the inference becomes more complex. This is because the hypothetical predicted genome size of the ancestor depends on both that of the outgroup and on the probability of change to a bigger or a smaller genome size. For example, if one extant species has genome size $\times$, another has size $2\times$, and the outgroup has $2\times$, it is simplest to infer that the ancestral genome size was $2\times$ and that this genome size was halved in one descendant lineage—as long as the probability of decrease equals the probability of increase. If the probability of increase is greater than or equal to twice the probability of decrease, it is just as simple to assume that the ancestral genome size was $\times$ and it then increased to $2\times$. Note that

LETTER TO THE EDITOR

the ancestral genome size cannot be measured directly; we can only evaluate how many other assumptions are required to satisfy a hypothesized change in size. If we hold that all ancestors had smaller genomes than virtually all descendants, we must assume that decreases in genome size are highly unlikely.

Making predictions about the genome sizes of ancestral species is only part of the challenge, however. In order to identify those ancestors, one must also have a reasonable idea of the phylogenetic relationships among extant species. Only then can we examine the ramifications of unidirectional changes in genome size by overlaying genome size data on the phylogeny. Fortunately, our understanding of plant phylogeny is increasing rapidly. This is particularly true for certain well-studied groups such as the grass family. Figure 1 shows the phylogenetic relationships among some grasses for which genome size is known.

Given the phylogeny and genome sizes for the extant species, we can then estimate the genome sizes of the ancestors under two different assumptions: an "increase-only" model, in which the inferred genome size of a hypothetical ancestor is no bigger than that of the smallest of its descendants, and an "agnostic model," which assumes that decreases in size are as likely as increases (i.e., that the direction of change is random).

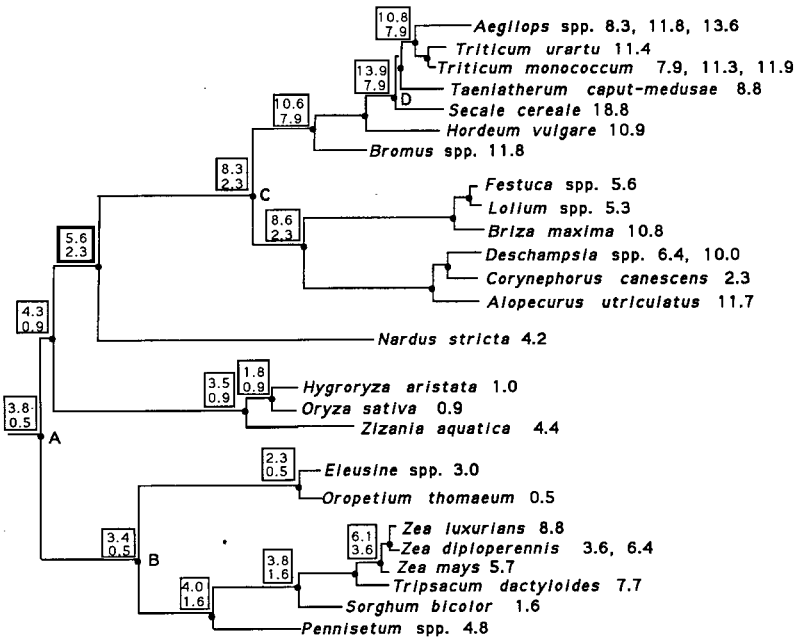

Figure 1. Phylogeny of Some Diploid Grasses for Which Genome Size Is Known.

Numerical values indicate pg DNA per 2C nucleus and are taken from Bennett and Smith (1976, 1991), Bennett et al. (1982), and Bennett and Leitch (1995). Note that some species have more than one value reported, and that we have included only species with diploid chromosome numbers to avoid complications caused by polyploidy. All of the hypothetical ancestors (nodes of the phylogenetic tree) are indicated by black dots. Nodes labeled A through D are discussed in the text. Branch lengths reflect the approximate number of changes. Phylogenetic relationships are based on the molecular analyses of Doebley (1990), Barker et al. (1995), Clark et al. (1995), and Kellogg et al. (1996). The numbers in the boxes at selected nodes represent inferences about ancestral genome size. The upper number assumes an agnostic model, in which size may decrease or increase and change is minimized; the lower number assumes that genome size can only increase.

Consider first the increase-only model (the lower numbers in the boxes in Figure 1). Three genome sizes have been reported for *Triticum monococcum*—7.9, 11.3, and 11.9 pg of DNA per diploid nucleus (Bennett and Leitch, 1995). Under the increase-only model, the ancestral genome size for *T. monococcum* must be no larger than 7.9 pg DNA/2C nucleus. Because *T. monococcum* and *T. urartu*, which are both A-genome wheats (Kimber and Feldman, 1987), are descended from a single ancestor, that ancestor must also have had 7.9 pg of DNA in its genome. Proceeding down the tree (i.e., back in time) toward node A (Figure 1), genome sizes can be assigned to all of the ancestors such that each one has a genome size equal to the smallest of those of its descendants. The same can be done for other branches of the tree. Upon examining these genome size estimates, we find that genome sizes have increased at least 28 times in the history of the grass family, that 14 of these changes must have been at least twofold increases, and of the 14, seven must have been at least threefold increases. Under this model, then, increases in genome size must not only be common, they must often be large. (Note that the best evidence indicates that the species included here are all diploids, so that these increases do not appear to have anything to do with polyploidy.)

What if genome sizes are as likely to decrease as they are to increase? This agnostic model, which makes no mechanistic assumptions, is usually preferred by phylogeneticists in the absence of any known process. The principle is that of Occam's Razor—explanations

LETTER TO THE EDITOR

should be no more complex than necessary to account for the data, and it is simplest to assume that ancestors had genome sizes similar to those of their descendants. Several algorithms that minimize the differences between hypothetical ancestors and extant ancestors have been developed (e.g., Farris, 1970; Swofford and Maddison, 1987; Maddison, 1991). Here, we use the algorithm of Maddison (1991), which minimizes the sum of the squared changes in genome size; this is basically a Brownian motion model, which implies that the direction of change is random and that amounts of change are normally distributed.

Values under the agnostic model are the upper numbers at each node in Figure 1. Following these estimates along the different branches leads to the inference of 29 increases and 26 decreases in genome size in the grass family, with four groups exhibiting both increases and decreases relative to their respective ancestors. This is a higher number of changes than the unidirectional model, but the total amount of change in genome size is smaller. Considering only changes of twofold or greater (as we did under the increase-only model), we would infer one decrease in the Aveneae (*Corynephorus*), one in the chloridoids (*Oropetium*), one in the panicoids (*Sorghum*), and one in the oryzoids (*Oryza*), but no large increases. However, even the agnostic model suggests that there was a steady increase between the hypothetical ancestor of the family (Figure 1; node A) and the hypothetical ancestor of *Secale* (Figure 1; node D).

One could quibble with this simplified example in many ways. Although all species included are diploids, it is possible that some of them contain small duplicated regions. Furthermore, not all groups of grasses are represented. Nonetheless, this simple exercise does allow us to evaluate the hypothesis that genomes can only get larger. For this to be so, we must be willing to accept 14 independent large (twofold or greater) increases in genome size, rather than four independent large decreases.

We must also believe that in the 70 million years since they shared a common ancestor (Figure 1; node A), the rice genome has only doubled in size and the sorghum genome has merely tripled. In the much shorter time since the divergence of *Pennisetum* and *Eleusine* (Figure 1; node B), one genome has increased in size by a factor of 10 and the other by a factor of six. And in the recent history of the genus *Festuca*, genome size has tripled in some species. If this is true, then the big question is not so much whether genome sizes can increase, but why those of sorghum and rice (for instance) have increased so little.

These two models have different implications for the frequency, amount, and direction of change in genome size. Moreover, the agnostic model assumes we know nothing about how changes in genome size might occur, whereas the increase-only model posits a ratchet or drive mechanism. Clearly, the agnostic model is not strictly appropriate, because there are known mechanisms whereby genome size can increase easily and rapidly. But whether we prefer the increase-only model depends on whether there are mechanisms through which plants may maintain and/or return to smaller genome sizes. Below, we discuss the possibility that plants are able to maintain a particular genome size and then discuss mechanisms that may lead to reductions in genome size.

We see two mechanisms that might lead to maintenance of genome size: resistance to retroelement amplification, and restricting contact with retroelements (i.e., precluding them from occupying a genome). These maintenance processes may be aided by selection, such that plants that become infected with retroelements and/or allow the elements to amplify are rapidly removed from the population.

It is likely that many plants are resistant to retroelement amplification. The various processes of transposable element and transgene suppression that have been documented in plants, many of which are associated with DNA methylation, all appear to be related to sequence repetition and all block the further amplification of repeated sequences. Indeed, suppression/methylation processes may have evolved as mechanisms for resisting virus infection and transposable element amplification (Bestor, 1990). Perhaps plants with small genomes have particularly effective repeat-induced gene silencing, which leaves transposable elements of all types at relatively low copy numbers.

Alternatively, perhaps plants with small genomes have been exposed to fewer retrotransposon families than have those with larger genomes. For example, in maize, five different families of highly repetitive retrotransposons comprise over 25% of the maize genome. Moreover, another ~25% of the genome may be made up of members of a predicted 1000 or more other families of lower copy–number retrotransposons (SanMiguel et al., 1996). Arabidopsis, despite having many classes of retrotransposons, does not have any interspersed elements with copy numbers greater than a dozen or so. It is not clear whether this is because Arabidopsis has never acquired (by either vertical or horizontal transmission) enough retrotransposons to have a reasonable chance that one might amplify to high copy numbers, or whether Arabidopsis carries the capacity to epigenetically inactivate all retroelements prior to their extensive amplification.

Selection against large genomes may reinforce mechanisms for retroelement silencing by eliminating from populations any organisms in which the mechanisms fail. Numerous studies have indicated that a smaller genome size correlates with such important biological phenomena as faster cell-cycle transition times, shorter generation times,

LETTER TO THE EDITOR

resistance to radiation, and optimum environment for growth (Sparrow and Miksche, 1961; Van't Hof and Sparrow, 1963; Bennett, 1972, 1976; Rayburn et al., 1985). If enough variation exists in genome size within a population, then different physiological outcomes could provide the raw material for natural selection. On the other hand, extensive DNA methylation of interspersed repetitive elements in plants (Bennetzen et al., 1994), and their low level or lack of expression (Avramova et al., 1995), indicate that plants keep repetitive retrotransposons largely silent, thereby minimizing their day-to-day effects on host biology. Hence, strong selective pressure for removal of these elements may not exist. Furthermore, the possible one-step removal of a single block of interspersed repeats (perhaps as much as 200 kb of DNA [Bennetzen et al., 1994]), by some unknown mechanism, would decrease the overall maize genome size by less than 0.01%. It is difficult to see how this could provide much of a selective advantage, unless the removal of this repetitive block altered the function of adjacent genes.

Although the mechanisms of retrotransposon amplification are clear and well characterized, a mechanism for the removal of substantial quantities of interspersed repetitive DNA has not been identified in plants (but see Petrov et al., 1996, for an example from Drosophila). Rapid changes in plant genome size have been noted, particularly in plants subjected to environmental stress, but the repetitive entities characterized in these studies were either tandem repeats or other repeats whose basic nature and interspersion patterns were not thoroughly investigated (Walbot and Cullis, 1985).

Despite the absence of a known mechanism that could substantially reduce nuclear DNA content in plants, our current understanding of plant genome organization does offer some insight into the possible mechanisms that an undiscovered genome shrinkage process may employ. First, because interspersed repetitive DNAs make up the majority of the repetitive DNA in complex plant genomes, the removal process would have to operate primarily on these sequences and would have to excise them without removing the adjacent plant genes. Similarly, many copies of the same retrotransposons are found in different repetitive blocks, and so unequal recombination between whole elements could, in theory, delete large blocks of repetitive DNA. However, these deletions would also include any genes between the blocks—the similarity of gene content and gene order within the grasses and other plants suggests that such gene deletions are rare, poorly transmitted, and/or strongly selected against (Bennetzen and Freeling, 1993; Moore et al., 1995).

Because many of these interspersed repeats are also found in large (and largely gene-free) heterochromatic blocks such as those near centromeres (Edwards et al., 1996), then it might be possible for unequal intrastrand recombination to remove these large blocks without resultant gene deletion. However, such unequal recombinations cause reciprocal deletions and duplications on the participating chromatids and, without selection, would be totally neutral vis-à-vis genome size. Such a mechanism, or any other deletional process that only removes interspersed repeats that are in large heterochromatic blocks, would lead to the prediction that small-genome plants and large-genome plants would largely differ in the size of centromeric heterochromatin but not in the size of their gene-rich (euchromatic) regions. However, comparative analyses of gene-containing regions of the maize, sorghum, and rice genomes indicate that it is the presence and size of repetitive blocks between genes that correlate with genome size (Avramova et al., 1996; Chen et al., 1997).

Second, the removal process must primarily target highly repetitive retroelements and not the middle repetitive retroelements with which they are intermixed, because middle repetitive retrotransposons are abundant both in large and small plant genomes.

Third, this hypothetical genome shrinking mechanism would have to be very active relative to amplification of mobile DNAs, because some closely related species (i.e., members of the same genus) can differ by more than fivefold in haploid genome size (Bennett and Leitch, 1995). Amplification can easily accomplish this genome size difference in just a few meiotic generations (although it probably does not under normal circumstances), and element removal would need to overwhelm this tide. Such an event might explain the large increase in genome size between nodes C and D (Figure 1), an increase that is postulated by both the increase-only and agnostic models.

To date, we have not observed any rapid decreases in interspersed repeat copy number, although gradual decreases in the copy number of an element may not have been detectable in these analyses. By contrast, rapid increases in retroelement copy numbers have been observed (Hirochika et al., 1996). It is possible, however, that unusual circumstances (e.g., severe stress) could induce both directed mechanisms of element removal or, more likely, strong selection against large genome size.

Unequal recombination between the two LTRs of a single element is one process for removing at least a portion of individual LTR-containing retrotransposons and has been routinely observed. Such excisions give rise to a solo LTR at the previous insertion site and a DNA circle that contains the element with a single LTR. The circle is presumably lost, thereby decreasing overall element copy number. However, detailed regional analyses in the DNA flanking *Adh1* of maize indicate very

LETTER TO THE EDITOR

few solo LTRs relative to intact elements (SanMiguel et al., 1996). This suggests that the insertion and resultant amplification of new element copies is much more common than is unequal recombinational removal of current copies, at least in maize. Moreover, plants (like other eukaryotes) tend to recombine primarily within genes, indicating that repetitive blocks should not be much affected by either equal or unequal recombination events.

Maize, however, with its large genome and numerous active transposons, may not be the best organism in which to find an operative mechanism of genome size decrease. Indeed, phylogenetic analyses point toward other species in which we might seek such a mechanism. The phylogenetic data can be interpreted as showing independent decreases in genome size in *Sorghum*, *Oryza*, *Oropetium*, and *Corynephorus*. Each of these species could be compared with its sister taxa across homologous regions of the genome in a manner similar to the comparisons performed between rice, sorghum, and maize by Chen et al. (1997). In these comparisons, patterns of nested retroelements or solo LTRs could potentially be detected.

The phylogeny is not only critical for determining the direction of change, that is, which taxa may have exhibited reductions in genome size, but also in identifying the appropriate sister taxa for comparison. The more closely related two species are, the easier it will be to detect sequence similarity among transposable elements, to identify solo LTRs, and to determine exactly which portions of the genome are present in the small-genome plants and absent in the large-genome ones. There is a direct analogy here with genetic studies. The direction of change in genetics is determined by keeping track of which plants are mutant and which are wild type. Likewise, the phylogenetic tree defines the direction of change. The appropriate comparisons in genetics are between the mutants and the inbred line in which the mutagenesis was done; this minimizes the confounding effects of genetic background. Similarly, the phylogenetic tree identifies the mutant (the taxon with the derived characteristic) and the species to which it is most closely related (most genetically similar).

That we have yet to identify a process through which repetitive elements may be removed from genomes does not indicate, of course, that such a mechanism is not present. Our understanding of complex plant genomes is still quite primitive, and we expect that many unanticipated phenomena will be brought to light in the next few years. Episodic strong selection against large genomes or an as-yet-undetected mechanism for the excision of interspersed retrotransposons could balance the genome expansion trend. The two of us disagree on the probability of finding such mechanisms. We do agree, however, that a mechanism that leads to rapid increases in genome size does exist. Thus, unless evidence for a comprehensive mechanism for removing interspersed repetitive DNAs is found, and/or strong selective pressures for reducing genome size can be determined, we must conclude that plants may indeed have a one way ticket to larger genome sizes.

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LETTER TO THE EDITOR

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