LETTER TO THE EDITOR

cooperation of cytoplasmic and mitochondrial lysyl-tRNA synthetases. EMBO J. **14,** 3461–3471.

Tsai, F.-Y., and Coruzzi, G.M. (1990). Dark-induced and organ-specific expression of two asparagine synthetase genes in *Pisum sativum.* EMBO J. **9,** 323–332.

Tsai, F.-Y., and Coruzzi, G.M. (1991). Light represses transcription of asparagine synthetase genes in photosynthetic and nonphotosynthetic organs of plants. Mol. Cell. Biol. **11,** 4966–4972.

van den Berg, C., Willemsen, V., Hage, W., Weisbeek, P., and Scheres, B. (1995). Cell fate in the root meristem is determined by directional signaling. Nature **378,** 62–65.

van den Berg, C., Willemsen, V., Hendriks, G., Weisbeek, P., and Scheres, B. (1997). Short-range control of cell differentiation in the *Arabidopsis* root meristem. Nature, in press.

van de Sande, K., Pawlowski, K., Czaja, I., Wieneke, U., Schell, J., Schmidt, J., Walden, R., Matvienko, M., Wellink, J., van Kammen, A., Franssen, H., and Bisseling, T. (1996). Modification of phytohormone response by a peptide encoded by *ENOD40* of legumes and a nonlegume. Science **273,** 370–373.

van Houdt, H., Ingelbrecht, I., Van Montagu, M., and Depicker, A. (1997). Post-transcriptional silencing of a neomycin phosphotransferase II transgene correlates with the accumulation of unproductive RNAs and with increased cytosine methylation of 3′ flanking regions. Plant J. **12,** 379–392.

Vaucheret, H., Nussaume, L., Palauqui, J.-C., Quillére, I., and Elmayan, T. (1997). A transcriptionally active state is required for post-transcriptional silencing (cosuppression) of nitrate reductase host genes and transgenes. Plant Cell **9,** 1495–1504.

Vernon, D.M., and Meinke, D.W. (1994). Embryogenic transformation of the suspensor in *twin*, a polyembryonic mutant of Arabidopsis. Dev. Biol. **165,** 566–573.

Vroemen, C.W., Langeveld, S., Ripper, G., Mayer, U., van Kammen, A., Jürgens, G., and de Vries, S.C. (1996). Pattern formation in the Arabidopsis embryo revealed by *position-specific lipid transfer protein* gene expression. Plant Cell **8,** 783–791.

Ward, B.M., Medville, R., Lazarowitz, S.G., and Turgeon, R. (1997). The geminivirus BL1 movement protein is associated with endoplasmic reticulum-derived tubules in developing phloem cells. J. Virol. **71,** 3726–3733.

Weig, A., Deswarte, C., and Chrispeels, M.J. (1997). The major intrinsic protein family of Arabidopsis has 23 members that form three distinct groups with functional aquaporins in each group. Plant Physiol. **114,** 1347–1357.

Zhang, J., and Somerville, C.R. (1997). Suspensor-derived polyembryony caused by altered expression of valyl-tRNA synthetase in the *twn2* mutant of Arabidopsis. Proc. Natl. Acad. Sci. USA **94,** 7349–7355.

Slow but Steady: Reduction of Genome Size through Biased Mutation

In their recent letter to the editor of THE PLANT CELL, Jeff Bennetzen and Elizabeth Kellogg (1997) grapple with the difficult and contentious issue of estimating the direction of genome size change during plant evolution. The authors argue that the frequency and magnitude of genome size changes in evolution cannot be directly ascertained by comparative phylogenetic methods. This is because doing so would require an a priori assumption about the relative likelihood of genome size increases compared to decreases, making the exercise circular. Even the "agnostic" model described by Bennetzen and Kellogg is a misnomer, because it is based on the assumption that genome size increases and decreases are equally likely. This constraint, which may or may not be valid, severely affects the outcome of their phylogenetic analyses.

Thus, we are left having to use our knowledge of genetic processes that affect genome size to guess how likely it is that the genome size would either increase or decrease during evolution. Bennetzen and Kellogg argue that although many sequences in eukaryotic genomes have a propensity to increase their copy number through transposition, thereby increasing genome size, there are no known mechanisms that counterbalance this inexorable trend toward "genomic obesity." Although I agree that retrotransposition increases genome size, I will argue that there exists a mechanism—biased spontaneous mutation—that can significantly reduce genome size over evolutionary time scales.

All studies of spontaneous mutation to date have shown that deletions are more frequent and longer than are insertions. For example, in mammals, deletions are three to seven times more frequent than are insertions and are, on average, somewhat larger (3.2 bp versus 2.4 bp; Graur et al., 1989). In Drosophila, the difference is even more profound—deletions are almost 10 times more frequent and almost seven times longer than are insertions (24.9 bp versus 3.2 bp; Petrov et al., 1996; Petrov and Hartl, 1997).

LETTER TO THE EDITOR

In both organisms, these biases in mutation frequency and size will lead to the progressive elimination of nonessential sequences. Admittedly, this process is very slow in mammals, where a pseudogene or a retrotransposon will take on average 884 million years (MYR) to lose half of its DNA. However, genome shrinkage is much faster in Drosophila, where the size of a nonessential piece of DNA will be reduced by half in only 15.4 MYR.

Both of these measurements were performed on animals, but there is no reason to believe that plants are different in their propensity to lose DNA through spontaneous mutation. Nevertheless, it remains to be determined whether plants generally lose DNA slowly, like mammals, or quickly, like Drosophila, and also whether different plant lineages vary widely in their rates of spontaneous DNA loss.

The problem with the argument of Bennetzen and Kellogg is that it gives a lot of weight to the experimental evidence that the genome size can increase quickly. This emphasis may not be warranted because although DNA addition through transposition can occur in rapid bursts, DNA loss through spontaneous deletion operates slowly, over tens of millions of years. Because our experiments are so short in duration (i.e., much shorter than 15 MYR), we are necessarily biased toward seeing only quick expansions of genomes and not noticing the contractions. However, over long periods of time, both of these processes will play a role. Given what we know today, it is impossible to assess if and where an equilibrium value will be reached.

One argument for the primary role of retrotransposition in changing genome size, and therefore for the predominance of genome size increases in evolution, is that large genomes tend to have more copies of retrotransposons. This argument would be valid if it were shown that the average size of nonessential sequences other than retrotransposons did not differ in small-genome versus large-genome lineages. In fact, the reverse appears to be the case. Where such assessments have been performed, they show that genome contractions or expansions affect all sequences that are free to vary in size. For example, bird introns are smaller than mammalian introns, which is consistent with birds having smaller genomes than mammals (Hughes and Hughes, 1995). Further evidence of indiscriminate genome shrinkage in birds comes from the observation that bird genomes are practically bereft of pseudogenes. This is in contrast to mammalian genomes, which often harbor tens or even hundreds of pseudogene copies per functional gene. Similarly, introns longer than 100 bp are significantly shorter in *D. melanogaster* compared to *D. virilis*, which is consistent with *D. virilis* having an almost twofold larger genome than that of *D. melanogaster* (E. Moriyama, D. Petrov, and D. Hartl, unpublished observations).

This correlation between genome and intron sizes, as well as the absence of pseudogenes in smaller genomes, can be explained by postulating that at least some variations in genome size are due to variation in the rate of DNA loss through spontaneous deletion. According to this model, the increased number of retrotransposons in large-genome lineages may be due in part to an increase in the number of possible nondeleterious insertion sites in the genome and to an increased time before each copy of a retrotransposon becomes unrecognizable due to multiple small deletions. Again, this argument is based on measurements carried out in animals, but there is no a priori reason to believe that plants are any different in this respect.

In conclusion, I believe that spontaneous deletions may provide a "return ticket" for some of the obese genomes, albeit on a different train. However, I also believe that the ultimate destination of these genome size trains remains unknown. Clearly, further studies combining a phylogenetic approach with estimates of genome size as well as investigations of the abundance of all types of nonessential DNA and of the patterns of spontaneous DNA loss through biased mutation are needed before we can hope to fully explain the "C-value" paradox.

Dmitri Petrov
Harvard University Society of Fellows
78 Mount Auburn Street
Cambridge, Massachusetts 02138
dpetrov@oeb.harvard.edu

REFERENCES

Bennetzen, J.L., and Kellogg, E.A. (1997). Do plants have a one-way ticket to genomic obesity? Plant Cell **9,** 1509–1514.

Graur, D., Shuali, Y., and Li, W.-H. (1989). Deletions in processed pseudogenes accumulate faster in rodents than in humans. J. Mol. Evol. **28,** 279–285.

Hughes, A.L., and Hughes, M.K. (1995). Small genomes for better flyers. Nature **377,** 391.

Petrov, D.A., and Hartl, D.L. (1997). Trash DNA is what gets thrown away: High rate of DNA loss in Drosophila. Gene, in press.

Petrov, D.A., Lozovskaya, E.R., and Hartl, D.L. (1996). High intrinsic rate of DNA loss in Drosophila. Nature **384,** 346–349.