Commentary

The 'bricolage' of the genome elucidated through evolutionary genomics

Geneticists are fond of referring to a genome as a 'book of life'. A genome contains all the information needed to create life: genes, the words of the book, are strung out on chromosomes, like 'beads on a string'. Although most would agree that this view of genomes is too simplistic, the fact remains that it has been a powerful metaphor. Recent discoveries, however, highlight just how unlike a book a genome seems to be, or at least how incredibly sloppy the publisher is: the book of life has many ostensibly extraneous letters, sections of duplicate paragraphs, and pages that simply do not seem to open.

When I came across the word 'bricolage' in Meagher and Vassiliadis' review in this issue of *New Phytologist*, I admit I had to look up the meaning of the word. Bricolage is the construction or creation of something from a diverse array of available objects, whatever might be at hand at the moment. Bricolage is a wonderful metaphor for evolution in general, and one that seems particularly appropriate for describing the evolution of genomes. In only the past few years, advances in computation and genetics have provided biologists with the data and means to begin understanding the organization, structure and history of genomes. From the beginning, evolutionary biology was generally recognized as being integral to the study of genomics (Eisen & Fraser, 2003). Evolutionary biologists are now just beginning to explore the evolution of genomes, but the bricolage nature of the genome means that this will likely not be an easy task. A diverse set of reviews on evolutionary genomics published in this issue of *New Phytologist* illustrates just how challenging the genome will be to understand. The reviews provide, however, some fascinating glimpses into what we know, and even more interesting, what we still do not know, about some very basic elements of genome evolution.

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Mitochondria: starting small

With a genome several orders of magnitude smaller than the nuclear genome and with a central role in primary metabolism, the mitochondrion seems like a genome that biologists would understand well. The endosymbiotic theory, and much genomic work, established the bacterial origin of the mitochondrion. Furthermore, the complete DNA sequence of the mitochondria of hundreds of eukaryotes have been in hand for many years. Mitochondria have few genes, and gene order tends to be highly conserved. It is almost dogmatic among biologists that mitochondria are transmitted from mother to offspring, that they do not recombine, and that every mitochondria in a cell is pretty much identical to any other mitochondria in that cell. Yet, we seem to have overestimated our knowledge of even this small organelle.

In their review, Barr *et al.* (pp. 39–50 in this issue) raise a number of questions about whether our assumptions about mitochondria need to be revised. Certainly, much of what we know about mitochondria is drawn from studies of animals, although, even there, these assumptions have been challenged (Ballard & Whitlock, 2004). Barr *et al.* broaden that challenge by reviewing what we know about transmission, recombination and heteroplasmy in plant and fungal mitochondria. Barr *et al.* make the case that exceptions to these rules could be critical in understanding mitochondrial evolution, because limited biparental transmission might allow for some recombination and therefore purging of deleterious mutations, while still allowing for the control of the spread of selfish genetic elements. Barr *et al.* point to a number of cases in plants of biparental transmission, recombination and heteroplasmy. For example, it has long been known that some conifers inherit mitochondrial DNA from both maternal and paternal parents. In addition, plant mitochondrial genomes seem to be different from other taxa that maintain heteroplasmy over generations by being able to rapidly expand substoichiometric, subgenomic molecules to normal levels. Although the mitochondrial genomes of plants and animals regularly seem to violate the normal assumptions, fungi provide an even more fertile arena for mitochondrial studies, as that group exhibits a wide variety of interesting means of mitochondrial transmission and regulation.

The duplicitous nuclear genome

The tree of life is undoubtedly rooted in a past of small genomes. Yet, today, some organisms, such as amoebae, salamanders and lilies, have enormous genomes with little seeming correspondence to the complexity of lifestyle (the *C*-value paradox).

More generally, do we know how genomes grow in size? Transposable elements clearly play an important role, accounting for a surprisingly large fraction of the genomes of many plants and animals alike (Kumar & Bennetzen, 1999). Chromosomal duplications and rearrangements also play a role in changing genome size (e.g. Arabidopsis Genome Initiative, 2000). More recent genomic analyses, however, have revealed a more startling and prominent role for whole-genome duplications, or polyploidy, than most biologists had imagined (Soltis, 2005 and references cited therein). Even the compact genome of the model plant, *Arabidopsis thaliana*, seems to have undergone three rounds of polyploidization in its evolutionary history (Vision *et al.*, 2000; Bowers *et al.*, 2003). Work done on a number of crop plants suggests that their genomes are also the result of either ancient or recent polyploidy events (Blanc & Wolfe, 2004a; Paterson *et al.*, 2004). To date, the genomic data seem to provide no reason not to expect that the genomes of most angiosperms (perhaps even most eukaryotes) will not have undergone some ancient polyploidy event.

In his review, Vision (pp. 51–59 in this issue) explores the consequences of gene duplication, both segmental and polyploidy, for comparing gene order across taxa and the evolution of novel genes. Clearly, novel genes can arise as the result of duplications. Unfortunately, comparative genomic studies are very much in their infancy, much of the work is descriptive and some of the basic computational tools, particularly gene annotation programs, have been called into question. For example, Vision describes a comparison of the complete genomes of rice and *Arabidopsis thaliana*: fully half of the genes predicted to occur in rice have no sequence similarity to genes in *A. thaliana*. However, 80% of the genes in the *A. thaliana* genome have some sequence similarity to genes found in rice. Bennetzen *et al.* (2004) argue that these putative novel rice genes are actually fragments of retrotransposons.

Despite the technical questions, comparative genomics has led to some fascinating observations on genome evolution. Although no review in this issue covered the subject, and although there has been some skepticism about its importance (Eisen & Fraser, 2003), horizontal gene transfer is likely to be another element of the bricolage of the genome. Any genome likely contains fragments from other, distant, or even unrelated, taxa (e.g. Mower *et al.*, 2004). Another fascinating example from Vision concerns the reduction of the genome of *A. thaliana*. Although *A. thaliana* has undergone three genome-wide duplication events, most genes in this species are present only in a single copy: the reduction in genome size gives an indication of how labile and dynamic the genome can be. More exciting is the observation that regulatory genes, such as transcription factors and signal transduction proteins, tend to be retained from large-scale duplications, whereas small-scale duplications tend to retain genes coding for secondary metabolism or those implicated in stress responses (Blanc & Wolfe, 2004b; Maere *et al.*, 2005). This observation has important implications for coevolution within the genome because it may lead to clustering of functionally related genes.

Clustered coadapted complexes

The idea that functionally related genes might be physically clustered in the genome as a 'coadapted gene complex' is an old one (Mayr, 1963): physical proximity would prevent recombination from breaking up advantageous combinations of loci. Genomics holds the promise of rigorously testing this hypothesis. Genomic association is especially important in the self-incompatibility system of the Brassicaceae where there must be complete linkage between genes controlling recognition and display of pollen genotype for proper function: a change in either pollen component or pistil component will lead to self-fertilization. In *Arabidopsis*, physical mapping shows that allelic *S*-locus regions are indeed organized into haplotypes, although, interestingly, the genes can be in different orders and orientations (Boyes *et al.*, 1997).

Charlesworth *et al.* (pp. 61–69 in this issue) review remarkable molecular and evolutionary genetic work on understanding how plants prevent self-fertilization (Rausher, 2005). The *S*-loci controlling non-self-recognition are incredibly polymorphic (e.g. Richman *et al.*, 1996). Generating this diversity seems an incredibly difficult puzzle as both recognition components must change and both must occur in the same haplotype to maintain self-incompatibility. Another mystery that genomics may solve is the origin of these non-self-recognition systems. Has some other molecular system, such as pathogen-recognition, been co-opted to form the molecular basis of self-incompatibility?

One's junk is another's treasure

Because genomes are filled with so-called 'junk DNA' as transposable elements and repetitive DNA, Meagher & Vassiliadis (pp. 71–80 in this issue) raise an interesting question in their review: what are the phenotypic consequences of repetitive DNA? Chromosome structure suggests that some repetitive DNA is functional at the genome level, perhaps functionally defining centromeres, telomeres and ribosomal genes. Recent work provides some intriguing suggestions that transposable elements might play a role in gene function and evolution. For example, Kumar & Bennetzen (1999) argue that retrotransposons can serve as *trans*-acting expression factors, controlling gene regulation.

More exciting possibilities have recently been suggested by genomic work. Jiang *et al.* (2004) reported that over 3000 transposable elements, called pack-MULEs, occur in the rice genome and that these carry fragments of cellular genes. Proteomic analysis indicated that some of these captured gene fragments might be functional. Even more exciting is the possible connection between copy number for a retrotransposon, BARE-1, in wild barley, and an environmental gradient in the Israeli

desert (Kalendar *et al.*, 2000). Local adaptation might be facilitated by selection on genome size or by selection on the possible physiological effects of individual BARE-1 insertion. Finally, Aminetzach *et al.* (2005) reported an adaptive transposable element insertion that conferred increased resistance to an organophosphate pesticide in *Drosophila melanogaster*. Clearly 'junk DNA' holds some hidden secrets.

Come over to the wild side

Finally, Rapp & Wendel (pp. 81–91 in this issue) present a review, which I found both exhilarating and chilling, where they explore epigenetics. Epigenetics is the study of heritable changes that occur without a change in the sequence of the DNA. A number of recent papers have raised the possibility that epigenetics may play an important yet underappreciated role in evolution (Rutherford & Lindquist, 1998; Queitsch *et al.*, 2002; Lolle *et al.*, 2005). Whether epigenetics is simply an interesting, but relatively unimportant, exception to the rules of genetics, or whether it represents a novel challenge to how organisms evolve, remains to be seen and much work needs to be done. The mechanisms of epigenetic change are now being rapidly uncovered, and include methylation, histone modification and a number of small RNAs (miRNA and siRNA). Even physical position on the chromosome can influence gene expression, because chromosomes have hot and cold spots for recombination (Copenhaver *et al.*, 1998).

Rapp & Wendel point out at least two examples where epigenetics might play an active role in evolution, and both examples involve genomic stress: polyploidy and hybridization. First, the salt marsh grass *Spartina* has hybridized twice in the last century and the hybrids have 'massive' methylation re-patterning compared with their ancestors (Salmon *et al.*, 2005). Second, the genus *Brassica* possesses an astonishing amount of morphological variation that may be epigenetically derived. Genetically identical allopolyploid lines were successfully selected for divergent for flowering time and nongenetic variation was implicated (Pires *et al.*, 2004). There even seems to be a connection between epigenetics and transposable elements: mechanisms of gene silencing may have evolved to repress foreign, invading DNA (Matzke *et al.*, 1999). Again, it remains to be seen how generally important epigenetics will be to understanding evolution, but there is clearly a connection between the genome and the environment that begs to be explored.

The future of evolutionary genomics

Contemplating the future of a field in its infancy seems rash, but I do have some thoughts. One of the most exciting aspects of evolutionary genomics is breaking free from the chains of genetic model organisms. Variation is the heart of evolutionary biology, and understanding evolution will require a catholic approach to the taxa we study, be they weeds or trees (Howe & Brunner, 2005; Mauricio, 2005a). The next steps of evolutionary genomics should move the field closer to a synthesis with evolutionary and ecological genetics. We now or will soon have the tools to begin an earnest search for within-species and within-population variation in various aspects of genome structure and epigenetics. Repetitive DNA, transposable elements and methylation states are all likely to vary at scales below that of species. Such variation should be subject to natural selection, like any other continuously varying trait. Finally, the tools of ecology should be brought to bear on questions of genome evolution (Mauricio, 2005b). Because the genome is a bricolage and not a very linear book, uncovering the mysteries of the genome will be difficult. However, this diverse set of reviews on evolutionary genomics shows us glimpses of an intriguing world of genome evolution.

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Mycorrhiza helper bacteria: a promising model for the genomic analysis of fungal–bacterial interactions

For a long time, the mycorrhizal symbiosis has been considered as a bipartite relationship between plant roots and mycorrhizal fungi. However, in natural conditions, mycorrhizas are surrounded by complex bacterial and fungal communities, which interact with the mycorrhiza–plant symbiosis at physical, metabolic and functional levels. That is why it is more relevant today to qualify mycorrhizal roots and associated microbial communities as a multitrophic mycorrhizal complex (Fig. 1; Frey-Klett *et al.*, 2005). Although it is quite clear that the mycorrhizal complexes play a major role in gross production and nutrient cycling, the structure and the functioning of these complexes, and more particularly the importance of the interactions between bacteria and the mycorrhizal symbiosis, have been so far very poorly documented. Seminal investigations of Bowen & Theodorou (1979) and then Garbaye & Bowen (1989) demonstrated that the rhizosphere microflora could have a positive or negative impact on the mycorrhizal symbiosis, depending on the bacterial isolates. Since that time, several studies have been conducted, on either endomycorrhizal or ectomycorrhizal symbiosis, to identify bacterial isolates promoting the mycorrhizal symbiosis, so-called 'mycorrhiza helper bacteria' (Garbaye, 1994). These helper bacteria belong to many bacterial groups and genera, such as Proteobacteria (*Pseudomonas*: Duponnois & Garbaye, 1991 and Founoune *et al.*, 2002; *Burkholderia*: Poole *et al.*, 2001; *Bradyrhizobium*: Xie *et al.*, 1995), Firmicutes (*Bacillus*: von Alten *et al.*, 1993 and Dunstan *et al.*, 1998; *Paenibacillus*: Budi *et al.*, 1999 and Poole *et al.*, 2001) and Actinomycetes (*Rhodococcus*: Poole *et al.*, 2001; *Streptomyces*: Schrey *et al.*, this issue, pp. 205–216).

'The challenge now is to monitor the kinetics of the expression of Amanita genes in the presence of different fungal-associated bacterial isolates, including mycorrhiza helper bacteria, not only in vitro but also in more natural conditions – in other words, in the presence of plant roots.'

