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The evolution of plant genomes — scaling up from a population perspective

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Plant genomes exhibit tremendous diversity in both their size and structure, with genome sizes across land plants ranging over two to three orders of magnitude and significant variation in structural organization was observed across species (EA Kellogg, JL Bennetzen, The evolution of nuclear genome structure in seed plants, *Am J Bot* 2004, 91:1709–1725). Five plant genomes are now either completely sequenced or in the draft stage; the grape (O Jaillon *et al*., The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla, *Nature* 2007, 449:463–467) and papaya (R Ming *et al*., The draft genome of the transgenic tropical fruit tree papaya (*Carica papaya* Linnaeus), *Nature* 2008, 452:991–997) whole genome sequences were reported most recently. Moreover, sequencing of 41 additional genomes is in progress. There is now an emerging consensus that understanding genome evolution requires consideration of the population genetics of genome diversification, and that description of evolutionary forces at the level of populations and within species can help identify the features that led to plant genome diversity (M Lynch, JS Conery, The origins of genome complexity, *Science* 2003, 302:1401–1404). In this review we focus on advances in our understanding of the mechanisms that drive the diversification of genomes. In particular, we look at the extent to which demographic features such as effective population size changes within species can drive genome evolution, discuss population genetic models of genome diversification associated with transposable element (TE) mobilization, and describe recent studies on the evolution of gene families.

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Population genetic models of genome diversification

Population genetic processes are governed to a large extent by a species' effective population size (Ne), and there has been recent theoretical work that suggests that changes in Ne play a key role in the evolution of genome composition and size [1,2,3••]. Population size changes affect the dynamics of genome evolution by altering the efficacy of natural selection. Under this nearly neutral model, reduction in Ne increases the probability of fixation of deleterious mutations [4], and genome-wide diversification thus proceeds largely via the accumulation of nonadaptive molecular changes [1]. Smaller population sizes, for example, are predicted to facilitate the passive accumulation of deleterious genome features, such as TE insertions, or nonsynonymous mutations in gene-coding sequences.

The extent to which genome evolution is driven by processes that are dependent on effective population sizes is unknown. Certainly, several features of genome structure have been shown to be consistent with this model over broad evolutionary timescales, including genome size, intron lengths, and transposon copy numbers [1,2,3••]. Reduced single nucleotide polymorphism (SNP) levels and large excesses of slightly deleterious variation are also observed in selfing species [5•,6,7••], consistent with the lower effective population sizes of inbreeding species compared to outcrossing taxa [8].

Despite these observations, however, there have been questions raised about the significance of the nearly neutral model for genome evolution both on theoretical [9] and empirical grounds [2,10,11]. Population and comparative genomic studies of closely related plant species offer an opportunity to evaluate population genetic theories of genome diversification [2,12], by examining the evolutionary consequences of these effects by comparing plant species with different life histories or comparing patterns of evolution across recombination gradients within genomes [5•,13,14].

There have been several comparative genomic studies contrasting *A. lyrata*, an outcrossing species, and the self-compatible *A. thaliana* that possesses a lower Ne [2]. There is little evidence, however, that nearly neutral mutations have accumulated at different rates in the genomes of these two species. An increased rate of protein evolution and a decrease in codon usage bias is not observed in *A. thaliana*, despite predictions of the nearly neutral model for these patterns in inbred species [12]. Moreover, the patterns of amino acid polymorphism and between-species divergence are indistinguishable between *A. thaliana* and *A. lyrata*; this is again inconsistent with the nearly neutral theory [15•]. Similar results were

obtained for a wider array of species comparisons [5•], although in this larger study there is some evidence for greater excesses of replacement polymorphism in selfing lineages.

The failure of the *Arabidopsis* comparison to yield results consistent with population genetic predictions has been attributed to a recent origin of selfing, as has also been similarly suggested for self-fertilizing species *C. elegans* [16]. Alternatively, unless there is a large class of mutations that are additive in their fitness effects, expectations based on Ne may be obscured by the masking of deleterious recessives in heterozygous, outcrossing taxa [17]. Clearly, there is a need to extend the analysis to a much broader array of taxonomic comparisons. These initial studies do suggest, however, that the patterns of genome structure and variation observed at large evolutionary timescales may not necessarily be evident in microevolutionary comparisons within and between closely related species. Bridging these two levels remains an important challenge in comparative genome studies.

Transposable elements and genome size

TEs comprise the major fraction of repetitive DNA in eukaryotes and appear to be responsible, in large part, for differences in genome sizes among species [18]. Retrotransposon insertions, for example, have been shown to account for the phenomenon of hybrid genome expansion in interspecific *Helianthus* hybrids [19•]. These hybrid species in this sunflower genus, which are not polyploid taxa, have up to 50% greater genome sizes than their progenitor species genomes, and *Ty3/gypsy* retroelement proliferation is responsible for between 60 and 80% of this size differential [19•]. Genome size evolution in diploid and allopolyploid *Gossypium* species also appear to be governed by differences in genomic insertion/deletion rates, including those attributable to TEs [20].

In plants, many TE families have existed over long evolutionary periods and experience eruptions of activity followed by phases of dormancy. Sequencing of 74 randomly selected BAC clones in *Zea mays*, for example, revealed a preponderance of LTR retrotransposons, and two major expansions of copy number in the last two million years that are partly responsible for the large genome size of maize [21]. In *Oryza australiensis*, a relative of Asian cultivated rice, bursts of transposition activity in the last three million years among three retrotransposon families (*RIRE1*, *Wallabi*, and *Kangourou*) resulted in the accumulation of ~90 000 element copies and a rapid twofold increase in genome size [22]. One of these families, *RIRE1*, also shows evidence for interspecific transfer to *O. australiensis* from other reproductively isolated *Oryza* species [23], although greater sampling of elements among these species is necessary to confirm the possibility of horizontal gene transfer.

TEs also appear to affect rates of gene fission in plants. Although the rates of gene fusion are similar in *O. sativa* versus *A. thaliana*, the rice genome has higher rates of gene splitting, correlated with the longer gene sequences in the former and the greater abundance of TEs [24]. While TEs represent key components, however, other genome features in plants also appear to be factors in long-term trends in genome size changes. In broad comparisons between *A. thaliana* and *O. sativa*, there does appear to be a net loss of ~10–13 introns in plant genomes for every 1 intron gains, suggesting erosion in genome size (and gene complexity) over large evolutionary timescales [25].

Population genetic models, transposons, and genome diversity

Much of the effort aimed at understanding the effects of TE activity on plant genome size has focused on mechanisms of transposition rate changes and recombination. Significantly less attention has been paid to how natural selection and insertion biases may limit the proliferation of TEs in plant genomes [26]. In metazoans, population genetic evidence suggests purifying selection against slightly deleterious TE insertions may be important in limiting the spread of TEs [27–29], which has been interpreted as evidence of a population size effect on the efficacy of purifying selection [30]. Evidence of lower population frequencies of *Ac-III* elements in *A. lyrata* compared with a neutral distribution observed in *A. thaliana* is also consistent with this view [31]. Further support is provided by the observation that MITE elements in the nonrecombining Y chromosome of *Silene latifolia* also segregate at considerably higher frequencies than on recombining chromosomes [32•].

While these observations reveal broad comparative patterns, determining the mechanisms by which TE accumulation (accompanied by the associated genome diversification) proceeds requires explicit tests of alternative evolutionary models. Two leading models describe, in part, how purifying selection can determine TE copy numbers and distribution [33]. The gene disruption model states that transposons are preferentially excluded from regions with high gene density because of disruptions of gene structure and regulation. The ectopic exchange model, in contrast, proposes that purifying selection eliminates TEs because of deleterious rearrangements caused by homologous recombination between identical, dispersed TE copies.

It has been argued based on theoretical grounds that recombinational ectopic exchange may not be an important force in limiting the accumulation of TEs in selfing species such as *A. thaliana* [2,34], and indeed

the correlation of *Arabidopsis* TE abundance with gene density in noncoding DNA appears to support the alternative deleterious insertion model [35]. There is some evidence, however, for the ectopic recombination model in *A. thaliana*. Under this model, the frequency of ectopic recombination is expected to operate more strongly to remove TE insertions in regions of high recombination, on larger TE families, and longer polymorphic element copies [36]. These predictions appear to fit a recent population survey of *Basho* elements in *A. thaliana*. Hollister and Gaut observed that longer TE copies segregate at lower frequency and persist for shorter periods than smaller elements [37]. *Basho* copy number may be especially likely to be subject to ectopic exchange because of their abundance (2% of the *A. thaliana* genome), large size (~2 kb), and high sequence similarity because of recent expansion of the family. There have also been observations in *O. sativa* that short elements tend to accumulate in regions of high recombination and long elements are over-represented in regions of low recombination that may also be attributed to the operation of ectopic exchange [38,39].

These results illustrate the advantage of a population genomic approach in testing evolutionary hypotheses on the specific evolutionary forces that drive genome diversification. Further study to test explicit predictions of these and other models may allow us to determine the precise factors that contribute to TE patterns that are so crucial to the evolution of genome size and structure (Box 1).

Gene duplication and polyploidy

Gene duplication is generally regarded as a major force in the origin of new genes and genetic functions, and gene families account for more than half of the genes in plant genomes. Population genetic models have provided a framework to study the fates of duplicated genes, including the partitioning of ancestral functions (subfunctionalization) or the evolution of new functions (neofunctionalization) [40], and data continue to accumulate on the extent to which these can explain the rate, patterns, and fates of duplicate gene evolution. In a recent study of 280 paralogous gene pairs, 85% of duplicate pairs in *A. thaliana* showed a pattern of expression change consistent with both subfunctionalization and neofunctionalization, with 45% of gene duplicates evolving complementary expression patterns [41•]. Neofunctionalization of duplicate genes also appears to result in novel gene organizations associated with metabolic function [42]. In both *Arabidopsis* and *Avena sativa*, it was shown that duplication followed by neofunctionalization and genome reorganization leads to the formation of operon-like gene clusters that contain coexpressed loci involved in triterpene biosynthesis [42].

There are now opportunities to examine the patterns of gene family evolution in the context of physical or genetic interaction networks associated with duplicated loci. This is clearly illustrated by a study of the MADS-box regulatory gene family, where phylogenetic analysis and interaction data from different plant taxa were investigated [43]. Although this gene family may have >110 members in extant angiosperms, these appear to have arisen from a set of 9–11 ancestral genes, and that rounds of whole genome duplications or polyploidization events have preserved the basic ancestral patterns of protein–protein interactions. In general, interactions between MADS-box proteins appear to be generally conserved across plant species, and the elaboration of this gene family through duplications is accompanied by increased interaction connectivity between subfamilies among MADS-box genes and shared interactors between gene duplicates [43].

This study of the MADS-box gene family and the role of whole genome duplication highlight continued interest in the fates of genes in polyploid genomes. One trend that is now emerging is that asymmetric gene retentions or losses between homeologous chromosomes following allopolyploidization appear to occur, as has been documented in grass species [44]. This asymmetry has been hypothesized to arise from transcriptional dominance of one homeologous genes over the others, a phenomenon that has been shown in a genome-wide scale in *A. arenosa* × *A. thaliana* hybrids [45]. There is also evidence for subfunctionalization of duplicated genes in polyploid *Gossypium* species [46••]. Cotton arises as an allopolyploid between two species with divergent genomes (the A and D genomes). In an assay of nearly 1500 homeologous genes in the domesticated polyploid taxa, 25–37% of expressed genes during seed trichome development shows biased gene

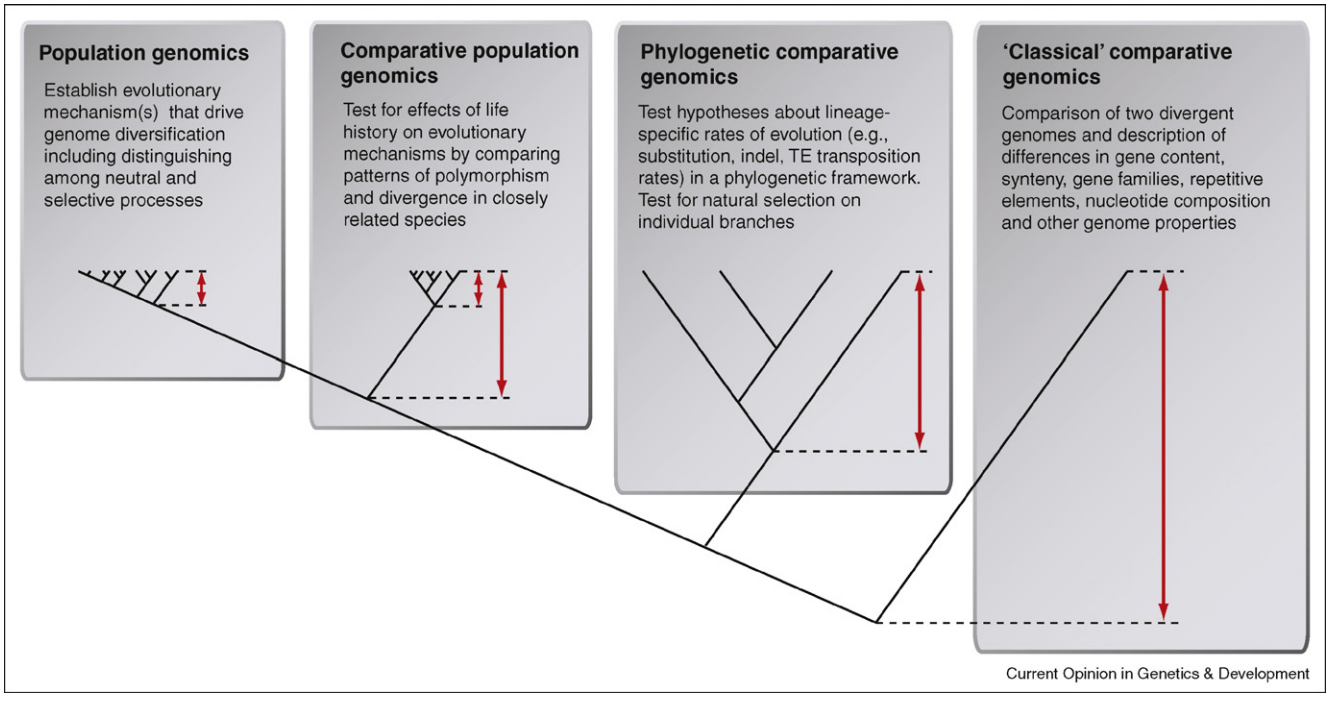
Box 1 Population genetic concepts and definitions

Effective population size (*Ne*) — the size of an idealized population that experiences genetic drift at the same rate as the population in question. Ne is affected by census size, level of inbreeding, population substructure, and other population and demographic attributes. Genomic regions can also differ in effective size because of the effects of background selection against deleterious mutations and selective sweeps of beneficial mutations localized at specific regions.

Nearly neutral theory — a model developed largely by Tomoko Ohta that proposes that a large fraction of mutations in the genome have a mildly deleterious or mildly advantageous effects on individual fitness. Slightly deleterious mutations are expected to segregate at low frequencies in populations, and to drift to fixation with higher probability in small populations owing to the effects of random genetic drift.

Ectopic recombination — crossing-over between dispersed regions of the genome that share sequence homology. The outcome of ectopic recombination may lead to chromosomal rearrangements.

Figure 1



Comparative genomics at different phylogenetic levels. 'Classical' comparative genomics utilizes two divergent genomes, such as rice and *Arabidopsis*, to make broad inferences about genome diversification. Phylogenetic comparative genomics has become an increasingly common means for determining molecular and evolutionary mechanisms of genome evolution in multiple closely related species (see e.g. [20]). Incorporating phylogeny provides a powerful means for determining lineage-specific effects on genome evolution. Comparative population genomics has been used in a few studies to examine the effects of life history on genome evolution (e.g. [15•]).

expression of either the A or D homeolog locus, and there is evidence for subfunctionalization in these polyploid duplicate genes [46••].

Conclusion

Genomic studies at various comparative levels — within populations, between closely related species and at higher taxonomic levels — have provided unprecedented insights into patterns of genome structure, and there are now concerted efforts to understand the evolutionary mechanisms that underlie this genome diversity (see Figure 1). Recent models to explain genome architecture appear to explain large-scale trends in plant genome evolution, but work in the last few years when comparing closely related species suggests that these models need to be extended or alternative models considered. Predictions on the effects of Ne on genome evolution, for example, are not met in comparative studies of closely related species with different life histories, both in plants and even animals [5•]. Understanding the causal basis of genome evolution will require continued explicit tests of models of DNA sequence evolution, TE proliferation and loss, and gene duplication. Analyses of large evolutionary trends and at microevolutionary timescales provide promising avenues to understand how genomes evolve and the evolutionary forces that drive their diversification.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Lynch M, Conery JS: **The origins of genome complexity**. *Science* 2003, **302**:1401-1404.

2. Wright SI, Nano N, Foxe JP, Dar VN: **Effective population size and tests of neutrality at cytoplasmic genes in *Arabidopsis***. *Genet Res* 2008, **90**:119-128.

3. Lynch M: *The Origins of Genome Architecture*. Sinauer; 2007.
An extended discussion on the population genetic forces that can affect patterns of genome structure and evolution.

4. Ohta T: **Near-neutrality in evolution of genes and gene regulation**. *Proc Natl Acad Sci U S A* 2002, **99**:16134-16137.

5. • Glemin S, Bazin E, Charlesworth D: **Impact of mating systems on patterns of sequence polymorphism in plants**. *Proc Royal Soc B* 2006, **273**:3011-3019.
A meta-analysis of available plant polymorphism sequence data that tested predictions about the effects of mating systems on molecular evolution.

6. Bustamante CD, Nielsen R, Sawyer SA, Olsen KM, Purugganan MD, Hartl DL: **The cost of inbreeding in *Arabidopsis***. *Nature* 2002, **416**:531-534.

7. •• Clark RM, Schweikert G, Toomajian C, Ossowski S, Zeller G, Shinn P, Warthmann N, Hu TT, Fu G, Hinds DA *et al.*: **Common sequence polymorphisms shaping genetic diversity in *Arabidopsis thaliana***. *Science* 2007, **317**:338-342.
The first comprehensive survey of nucleotide variation across the entire genome in a plant population.

8. Charlesworth B, Morgan MT, Charlesworth D: **The effects of deleterious mutations on neutral molecular variation**. *Genetics* 1993, **134**:1289-1303.

9. Charlesworth B, Barton N: **Genome size: does bigger mean worse?** *Curr Biol* 2004, **14**:R233-R235.

10. Vinogradov AE: **Testing genome complexity**. *Science* 2004, **304**:389-390.

11. Gregory TR, Witt JDS: **Population size and genome size in fishes: a closer look**. *Genome* 2008, **51**:309-313.

12. Wright SI, Lauga B, Charlesworth D: **Rates and patterns of molecular evolution in inbred and outbred *Arabidopsis***. *Mol Biol Evol* 2002, **19**:1407-1420.

13. Gaut BS, Wright SI, Rizzon C, Dvorak J, Anderson LK: **Recombination: an underappreciated factor in the evolution of plant genomes**. *Nat Rev Genetics* 2007, **8**:77-84.

14. Wright SI, Foxe JP, DeRose-Wilson L, Kawabe A, Looseley M, Gaut BS, Charlesworth D: **Testing for effects of recombination rate on nucleotide diversity in natural populations of *Arabidopsis lyrata***. *Genetics* 2006, **174**:1421-1430.

15. • Foxe JP, Dar VUN, Zheng H, Nordborg M, Gaus BS, Wright SI: **Selection on amino acid substitutions in *Arabidopsis***. *Mol Biol Evol* 2008, **25**:1375-1383.
First study to compare polymorphism and divergence data for a large number of genes from two closely related plant species.

16. Cutter AD, Wasmuth JD, Washington NL: **Patterns of molecular evolution in *Caenorhabditis* preclude ancient origins of selfing**. *Genetics* 2008, **178**:2093-2104.

17. Glemin S: **Mating systems and the efficacy of selection at the molecular level**. *Genetics* 2007, **177**:905-916.

18. Hawkins JS, Grover CE, Wendel JF: **Repeated big bangs and the expanding universe: directionality in plant genome size evolution**. *Plant Sci* 2008, **174**:557-562.

19. • Ungerer MC, Strakosh SC, Zhen Y: **Genome expansion in three hybrid sunflower species is associated with retrotransposon proliferation**. *Curr Biol* 2006, **16**:R872-R873.
Evidence of how retrotransposon activity increases after interspecific hybridization, leading to enlarged genomes in hybrid species.

20. Grover CE, Yu Y, Wing RA, Paterson AH, Wendel JF: **A phylogenetic analysis of indel dynamics in the cotton genus**. *Mol Biol Evol* 2008, **25**:1415-1428.

21. Liu R, Vitte C, Ma J, Mahama AA, Dhliwayo T, Lee M, Bennetzen J: **A GeneTrek analysis of the maize genome**. *Proc Natl Acad Sci U S A* 2007, **104**:11844-11849.

22. Piegu B, Guyot R, Picault N, Roulin A, Saniyal A, Kim H, Collura K, Brar DS, Jackson S, Wing RA, Panaud O: **Doubling genome size without polyploidization: dynamics of retrotransposition-driven genomic expansions in *Oryza australiensis*, a wild relative of rice**. *Genome Res* 2006, **16**:1262-1269.

23. Roulin A, Piegu B, Wing RA, Panaud O: **Evidence of multiple horizontal transfers of the long terminal repeat retrotransposon *RIRE1* within the genus *Oryza***. *Plant J* 2008, **53**:950-959.

24. Nakamura Y, Itoh T, Martin W: **Rate and polarity of gene fusion and fission in *Oryza sativa* and *Arabidopsis thaliana***. *Mol Biol Evol* 2007, **24**:110-121.

25. Roy SW, Penny D: **Patterns of intron loss and gain in plants: intron-loss dominated evolution and genome-wide comparison of *O. sativa* and *A. thaliana***. *Mol Biol Evol* 2007, **24**:171-181.

26. Lin X, Kaul S, Rounsley S, Shea TP, Benito M-I, Town CD, Fujii CY, Mason T, Bowman CL, Barnstead M *et al.*: **Sequence and analysis of chromosome 2 of the plant *Arabidopsis thaliana***. *Nature* 1999, **402**:761-769.

27. Duvernell DD, Turner BJ: **Variation and divergence of death valley pupfish populations at retrotransposon-defined loci**. *Mol Biol Evol* 1999, **16**:363-371.

28. Batzer MA, Deininger PL: **Alu repeats and human genomic diversity**. *Nat Rev Genetics* 2002, **3**:370-379.

29. Charlesworth B, Langley CH: **The population genetics of Drosophila transposable elements**. *Annu Rev Genet* 1989, **23**:251-287.

30. Neafsey DE, Blumenstiel JP, Hartl D: **Different regulatory mechanisms underlie similar transposable element profiles in pufferfish and fruitflies**. *Mol Biol Evol* 2004, **21**:2310-2318.

31. Wright SI, Quang HL, Schoen DJ, Bureau TE: **Population dynamics of an Ac-like transposable element in self- and cross-pollinating *Arabidopsis***. *Genetics* 2001, **158**:1279-1288.

32. • Bergero R, Charlesworth D: **Active miniature transposons from a plant genome and its nonrecombining Y chromosome**. *Genetics* 2008, **178**:1085-1092.
This study demonstrates that Y-linked MITEs segregate at substantially higher frequency than those found on autosomes consistent with a relaxation of purifying selection in nonrecombining regions.

33. Nuzhdin SV: **Sure facts, speculations, and open questions about the evolution of transposable element copy number**. *Genetica* 1999, **107**:129-137.

34. Wright SI, Schoen DJ: **Transposon dynamics and the breeding system**. *Genetica* 1999, **107**:139-148.

35. Wright SI, Agrawal N, Bureau TE: **Effects of recombination rate and gene density on transposable element distributions in *Arabidopsis thaliana***. *Genome Res* 2003, **13**:1897-1903.

36. Petrov DA, Aminetzach YT, Davis JC, Bensasson D, Hirsh AE, Size matters: **Non-LTR retrotransposable elements and ectopic recombination in *Drosophila***. *Mol Biol Evol* 2003, **20**:880-892.

37. Hollister JD, Gaut BS: **Population and evolutionary dynamics of Helitron transposable elements in *Arabidopsis thaliana***. *Mol Biol Evol* 2008, **24**:2515-2524.

38. International Rice Genome Sequencing Consortium: **The map-based sequence of the rice genome**. *Nature* 2005, **436**:793-800.

39. Gaut BS, Ross-Ibarra: **Selection on major components of angiosperm genomes**. *Science* 2008, **320**:484-486.

40. Force A, Lynch M, Pickett FB, Amores A, Yan YL, Postlethwait J: **Preservation of duplicate genes by complementary, degenerative mutations**. *Genetics* 1999, **151**:1531-1545.

41. • Duarte JM, Cui L, Wall PK, Zhang Q, Zhang X, Leebens-Mack J, Ma H, Altman N, dePamphilis CW: **Expression pattern shifts following duplication indicative of subfunctionalization and neofunctionalization in regulatory genes of *Arabidopsis***. *Mol Biol Evol* 2006, **23**:469-478.
A systematic analysis of the fates of duplicated genes in a plant genome, demonstrating the evolution of subfunctionalization and neofunctionalization.

42. Field B, Osbourn AE: **Metabolic diversification — independent assembly of operon-like gene clusters in different plants**. *Science* 2008, **320**:543-547.

43. Veron A, Kaufmann K, Borrnberg-Bauer E: **Evidence of interaction network evolution by whole genome duplications: a case study in MADS-box proteins**. *Mol Biol Evol* 2007, **24**:670-678.

44. Kim H, Huritz B, Yu Y, Collura K, Gill N, SanMiguel P, Mullikin JC, Maher C, Nelson W, Wissotski M: **Construction, alignment and analysis of twelve framework physical maps that represent the**

ten genome types of the genus *Oryza*. *Genome Biol* 2008, **9**:R45.

45. Hazzouri KM, Mohajer A, Dejak SI, Otto SP, Wright SI: **Contrasting patterns of transposable element insertion polymorphism and nucleotide diversity in autotetraploid and allotetraploid *Arabidopsis* species**. *Genetics* 2008, **179**:581-592.

46. Hovav R, Udall JA, Chaudhary B, Rapp R, Flagel L,
•• Wendell JF: **Partitioned expression of duplicated genes during development and evolution of a single cell in a polyploid plant**. *Proc Natl Acad Sci U S A* 2008, **105**:6191-6195.

A large-scale survey of the expression patterns of duplicate genes following polyploidy genome formation.