Reconstruction of monocotelydeneous proto-chromosomes reveals faster evolution in plants than in animals

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Paleogenomics seeks to reconstruct ancestral genomes from the genes of today’s species. The characterization of paleo-duplications represented by 11,737 orthologs and 4,382 paralogs identified in five species belonging to three of the agronomically most important subfamilies of grasses, that is, Ehrhartoideae (rice) Panicoideae (sorghum, maize), and Pooidaeae (wheat, barley), permitted us to propose a model for an ancestral genome with a minimal size of 33.6 Mbp structured in five proto-chromosomes containing at least 9,138 predicted proto-genes. It appears that four major evolutionary shuffling events (α, β, γ, and δ) explain the divergence of these five cereal genomes during their evolution from a common paleo-ancestor. Comparative analysis of ancestral gene function with rice as a reference indicated that five categories of genes were preferentially modified during evolution. Furthermore, alignments between the five grass proto-chromosomes and the recently identified seven eudicot proto-chromosomes indicated that additional very active episodes of genome rearrangements and gene mobility occurred during angiosperm evolution. If one compares the pace of primate evolution of 90 million years (233 species) to 60 million years of the Poaceae (10,000 species), change in chromosome structure through speciation has accelerated significantly in plants.

Results

Cereal Genome Synteny and Duplication Pattern. By using alignment parameters and statistical tests described in ref. 15 we analyzed the syntenic relationships between the rice, maize, sorghum, wheat, and barley genomes using various resources as described in SI Appendix. Using rice as a reference genome with 41,046 gene models, we identified 4,454 maize orthologs (defining 30 syntenic blocks), 6,147 sorghum orthologs (12 syntenic blocks), and 827 wheat orthologs (13

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tetraploidization of the genome. In total, 11,737 orthologous pairs were identified between the rice, maize, sorghum, wheat, and barley genomes. Each line represents a duplicated gene. The different colors reflect the origin from the five ancestral proto-chromosomes (15). (C) Distribution of the average CIP/CALP values observed for the orthologous (colored bars) and paralogous (colored curves) genes in the five cereal genomes. The number of genes in each category (paralogous vs. orthologous) is displayed within five classes (from 70 to 100%) of average CIP/CALP values. The asterisks indicate lineage specific events that affect the distribution, that is, the w1-w5-w7 translocation in wheat, the recent r11-r12 duplication in rice and the tetraploidisation (WGD) in maize. A time scale is provided on the left side of the diagram.

Two methods can be used for the in silico identification and characterization of gene duplications. The most robust and direct approach, called “intragenome duplication” (ID), consists in aligning a genome sequence against itself with stringent alignment criteria and statistical validation. We have used it recently to initiate paleogenomics studies in grasses (15). The second indirect approach, called “double synteny” (DS) or “doubly conserved synteny” (DCS), is based on the identification of chromosomal duplications through the detection of regions showing a high proportion of gene matches on two different chromosomes within a genome and corresponding to two syntenic regions in another genome (for review, see ref. 13). In this study, we reassessed duplications in the rice, maize, sorghum, wheat, and barley genomes through a combination of ID and DS approaches with the stringent alignment parameters defined in Salse et al. (15) (SI Appendix). Ten (383 paralogous), 17 (3,469 paralogous), 8 (390 paralogous), 10 (102 paralogous), and 9 (38 paralogous) intragenomic duplications were identified and characterized in the rice, maize, sorghum, wheat, and barley genomes, respectively (Fig. 1B and Fig. S2 in SI Appendix). In total, 54 interchromosomal duplications were characterized individually in the five cereal genomes, compared with the 31 previously identified in the rice (18), sorghum (19), barley (20), maize (16), and wheat (21) genomes using the DS approach, illustrating the advantage of combining the two methods. They represent 76%, 83%, 82%, 73%, and 75% of the rice, maize, sorghum, wheat, and barley genomes, respectively. Thus, in total, 4,382 paralogous genes were identified for the five cereal genomes providing the largest set of conserved duplicated genes in cereals to date (13, 15).

Integration of the independent analyses of the duplications within and synteny between the 5 major cereal genomes [hereafter referred to r for rice, m for maize, s for sorghum, and t for the Triticeae (wheat and barley)] allowed us to characterize them more precisely the seven shared duplications identified recently among the ancestral grass chromosomal groups (15, 22). These paleo-duplications were found on the following chromosome pair combinations: t4-t5/r11-r12/s5/s8/m2-m4-m1-m3-m10, t1-t3/r5-r1/s9/s3/m6-m8-m3, t1-t4/r10-r3/s1/m1-m5-m9, t2-t4/r7/r3/s2/m2-m7-m1-m9-m5, t2-t6/r2/s6-s4/m2-m10-m4-m5, t5-t7/r9-r8/s2-s7/m2-m7-m1-m4-m10-m6, and t6-t7/r2-r6/s4-s10/m4-m5-m6-m9 (Table S1 in SI Appendix). Sequence similarity comparisons (SI Appendix) of the 11,737 orthologous and 4,382 paralogous gene pairs identified in the five species clearly confirmed the coexistence within each genome
of ancestral shared duplications and recent lineage-specific duplications (Fig. 1B). Analysis of the distribution of sequence similarity between all orthologous gene pairs (Fig. 1C, bars) showed a peak for average CIP/CALP values of 85–80% which reflects the speciation of the five genomes from a common ancestor 50–70 mya. When the distribution of sequence similarity between paralogous genes is compared, two peaks are observed (Fig. 1C, curves). The first one (average CIP/CALP value of 85–80%) overlaps with the speciation of the 5 genomes from a common ancestor 50–70 mya thereby reflecting the ancestral shared duplications whereas, the second peak (average CIP/CALP value of 100–95%) is a result of lineage-specific and recent duplications such as the r1-r2 duplication in rice and the maize tetraploidisation (Fig. 1C).

To support the use of the comparative analyses in genetic mapping, we developed a user-friendly online Web tool called “Narcisse-Cereals” based on the public “Narcisse” platform (23) that allows us to visualize the 11,737 orthologs and the 4,382 paralogs characterized in the five cereal genomes (www.clermont.inra.fr/umr1095/narcisse_cereals) as well as gain access to the raw data (gene name, sequence, position, and alignment criteria) obtained from the analysis of the synteny and duplication of the rice, maize, sorghum, wheat, and barley genomes.

**Cereal Genome Ancestor Structure and Function.** The integration of rice, maize, and sorghum whole genome sequences in the comparative analysis allowed us to further characterize the ancestral genome structure in terms of size and gene content. Reconstruction of the five proto-chromosomes structural content was performed following the approach described by Murphy et al. (6) and Nakatani et al. (10) in the mammalian and early vertebrate paleogenomics studies, respectively, in which orthologous chromosomal segments are compared systematically in dot plots (Fig. 2). Here, the dot plot graph theory approach was applied by taking into account the syntenic as well as the shared and lineage specific duplications described above (SI Appendix). A set of 12 dot plots was first created for each orthologous chromosomal segment, and these were then assembled by the combination of two or three blocks into five nonredundant sets of duplicates that correspond to the five proto-chromosomes (Fig. 2 and Fig. S3 in SI Appendix). Despite numerous rearrangements (Fig. 2), a sufficient number of gene pairs remained that allowed us to identify large syntenic blocks, shared between the three genomes and derived (diagonal dot plots) from the common ancestor with five proto-chromosomes. We then estimated the ancestral or minimum gene number by calculating the number of genes that are conserved between at least two genomes. Moreover, the size of the ancestral genome was estimated on the basis of the longest shared sequence conserved between at least two genomes. In both cases, knowledge about the regions corresponding to the seven ancestral shared duplications (gray areas in Fig. 2 and Table S1 in SI Appendix) allowed us to eliminate redundancy and provide a robust ancestor model (14). For example, the first proto-chromosome (ancestral chromosome A5) exemplifies the synteny between r5-s9-m6/8 (first dot plot) and r1-s3-m3/8 (second dot plot) as well as the paleo-duplication identified between r5-r1, s9-s3, and m6/8-m3/8 (represented with a gray block) (15). For this chromosome, the dot plot analysis revealed seven regions including the centromere (Fig. 2) that are delimited by the conserved duplication boundaries. These blocks were then used to estimate the number of conserved genes between rice and maize (blue and green) and, rice and sorghum (red). This resulted in a final set of 2,145 nonredundant ancestral genes located within seven chromosomal regions on proto-chromosome A5 (Fig. 2). If one considers the sum of the length for the 2,145 conserved CDSs to calculate the minimal size for this chromosome (with 3.8 kb per gene on average), then proto-chromosome A5 was at least 6.3 Mb in size. Using the same approach, we analyzed the four other proto-chromosomes and identified 563 genes/2.8 Mb, 1,083 genes/4.1 Mb, 2,754 genes/9.3 Mb, and 2,639 genes/11.1 Mb for proto-chromosomes A11, A8, A4, and A7, respectively (Fig. 2). Thus, we conclude that the ancestral genome with five proto-chromosomes contained at least 9,138 proteogenes (located on 22 proto-chromosome blocks) representing a minimum size of 33.6 Mb. Here, the ancestor genome size is considered as a minimal size corresponding to the cumulative “gene space” free of transposable elements (TEs) or repeated elements. Of course, we cannot exclude the presence of TEs in the cereal ancestor. However, it is not possible to estimate their amount based on comparative analyses because of high transposition activity and rapid turnover of TEs in the cereal genomes (14) which only allow the detection of less than 4 million years old insertions through intraspecific comparisons (24).

To assign potential function to the 9,138 ancestral genes and identify those that were modified preferentially during evolution, we selected a dataset consisting of 65 gene ontology (GO) classes...
associated with the 41,046 rice gene models (http://gnn.tigr.org/tdb/e2kl/osal1/GO.retrieval.shtml) and compared it with the relative distribution of gene ontology in the 9,138 ancestral gene set. A two-sample χ² test of proportions was performed with the number of genes observed in each of the 65 GO classes between the rice and the ancestor gene contents. A P value >10⁻⁵ was found for 28 GO classes and was considered to reflect the under-representation of these categories between the rice and ancestor genomes. Among them, five classes (corresponding to transcription factor activity, transcription, biological process, DNA binding, and structural molecule activity) were underrepresented in the ancestor compared with the rice genome gene content (Fig. S4 in SI Appendix). This suggests that genes in these five classes were affected particularly by ancestral and lineage specific rearrangements (mainly duplications) that resulted in additional copies potentially providing a selective advantage during evolution and adaptation.

Acceleration of Evolution of Flowering Plants. To reconstruct the rice, maize, sorghum, wheat, and barley genomes from the five ancestral proto-chromosomes containing 9,138 genes, we propose an evolutionary model that involves 4 major (α, β, δ, and γ) events, including the nomenclature defined in studies of the A. thaliana genome evolution (25). Before the divergence of the five grass genomes, the ancestor with five proto-chromosomes underwent a whole genome duplication (WGDD) that resulted in n = 10 chromosomes intermediate (δ event, Fig. 3A). After this tetraploidization, 2 interchromosomal translocations and fusions (γ event) resulted in two new chromosomes and an n = 12 intermediate ancestor (Fig. 3A). The Panicoideae have evolved from this ancestral 12 chromosomes genome after two chromosomal fusions (β event) that resulted in an intermediate ancestor with n = 10 chromosomes (Fig. 3A). Then, maize and sorghum evolved independently from this ancestor. Whereas the sorghum genome structure remained similar to the ancestral genome, maize underwent a lineage-specific whole genome duplication (α event) that produced an intermediate with n = 20 chromosomes. Rapidly, 17 translocations and chromosome fusions led to a final number of 10 chromosomes (Fig. 3A). The α event corresponds to the tetraploidization described in previous studies (15). In this model, the Ehrhartioideae have retained the original 12 chromosomes and rice underwent lineage-specific rearrangements with recent duplications between the r11 and r12 chromosomes (α events in Fig. 3A). Finally, from the intermediate-ancestral genome with 12 chromosomes, the Pooideae underwent 5 chromosomal fusions that resulted in an ancestral Triticeae genome with n = 7 chromosomes (β event in the Fig. 3A). The Triticeae (wheat, barley, and rye), represented as a single genome in Fig. 3A, have retained the seven chromosomes as a basic chromosome number and underwent additional minor polyploidization events, segmental duplications, and translocations. For example, our analysis clearly established that a translocation between chromosomes 4 and 5 is common to wheat and barley whereas the previously reported translocation between chromosomes 4–7 is shared between the wheat and the rye genome only (15) (Fig. 3A).

Recent paleogenomics analyses within the eudicot family (11, 26, 27) led to two models illustrated in Fig. 3A. In the first one, the grape, Arabidopsis, and poplar genomes derive from a hexaploid ancestor with seven proto-chromosomes that underwent one and two specific whole genome duplications in poplar and Arabidopsis, respectively, whereas the grape remained unduplicated (11, 26, 27). In the second scenario, the eudicot genomes derive from a tetraploid ancestor with 7 proto-chromosomes that underwent specific whole genome duplications in the poplar, grape, and Arabidopsis lineages (12). Here, we wanted to exploit knowledge about the eudicots and monocots proto-chromosomes structures to see whether we can increase our understanding of the events that led to the divergence between these two main plant lineages. The five monocots proto-chromosomes (with 9,138 genes) were aligned with the seven eudicots proto-chromosomes [with 9,731 genes; Jaillon et al. (11)] using the approach describe previously for the identification of orthologs between the five cereal genomes. The results (Fig. 3B) show no orthologous chromosomal relationships between the five monocots proto-chromosomes (or chromosome arms or even chromosome blocks) and the seven eudicots proto-chromosomes. This indicates clearly that macrocolinearity has been largely eroded since the two major groups of angiosperms diverged from a common ancestor 150–300 mya and that the lack of colinearity observed previously between the monocot and dicot genomes was not due to a limited dataset or statistical methods but really reflects an active history of rearrangements during the evolution of the plant genomes.

Discussion

Paleoecology of the Monocot Chromosome Structural Evolution. As the number of sequenced genomes grows, paleogenomics is becoming an increasingly important research area that provides insights into plant and animal genome evolution. By combining new alignment criteria and statistical validation that take into account the gene position information, we improved the characterization of paralogous or orthologous gene pairs previously identified in cereals with less stringent methods such as orthoMCL (28), INPARANOID (29) and MCScan (26). The conserved gene constitute syntenic blocks (characterized by the distance between two genes and the number of genes within a block) that then can be efficiently used to infer ancestral relationships even in the absence of complete contiguous genome sequences, such as barley and wheat. When taking this analysis into the perspective of parallel evolution of the plant and animal kingdoms, our data suggest that plants genomes were affected by more rapid changes in chromosomal architecture and frequency of manifesting these changes in speciation than mammalian genomes. We would attribute these features to the evolution of DNA replication and repair mechanisms in plants that have to account for the immobility of plants versus animals and their vulnerability to environmental changes.

We show that overall, among 73% of homologous genes identified between the five cereal genomes (i.e., 27% of species-specific genes, so called orphans, or possibly artefacts of genome annotation), only 12.5% are still conserved at orthologous positions (i.e., 87.2% of gene transposition) after 50–70 my of evolution, demonstrating a high rate of gene translocation in these genomes. This is a much higher rate than previously reported (<50%) with smaller datasets (30), which is likely due to the conservative approach we have used in this study or inflated gene counts in previous whole genome annotation projects. In any case, polyploidization (either as part of whole genome duplications or genome hybridizations) and the degree of gene copying events appear to be major factors involved in the deterioration of syntenic relationships in plant genomes (31). Freeing et al. (32) reported recently that up to 75% of the genes in Arabidopsis transposed after the origin of the Brassicaceae, compared with 87.2% estimated in the current analysis. The authors suggest that negative (i.e., purifying) selection may remove transposed copies of members of some gene families preferentially, whereas positive selection favors transposition of copies from other gene families. Our data confirm these observations and support the hypothesis that genome plasticity resulting from high gene transposition frequency offers the opportunity to positively select useful physical gene interaction considered selectively advantageous and to remove any other combinations considered selectively deleterious.

The characterization of the largest number of orthologs (11,737) and paralogs (4,382) within a single analysis across five cereal genomes and the identification of shared (seven paleo-duplications) and lineage specific duplications, allowed us to describe precisely four successive evolutionary events, δ (whole genome duplication event), γ (cereal ancestor shuffling events), β (cereal ancestor intermediate shuffling events), and α (lineage-specific shuffling
events) that have shaped the grass genomes during the 50–70 my of evolution from a five proto-chromosomes ancestor. Such fundamental evolutionary mechanisms have been revealed also through recent studies in vertebrates (10) to 13 proto-chromosomes more than 400 mya) (10) and in the dicotyledons (seven protruchromosomes 120 mya) (11). Interestingly, both in animal and plants, similar evolutionary mechanisms have been described with a reduced number of proto-chromosomes and several rounds of WGD followed by lineage-specific rearrangements leading to different chromosome numbers in today’s species. Although there are many similarities among the eukaryotic kingdoms with respect to the characteristics of such chromosomal rearrangements, there are also significant differences. Polyploidization, a dominant force in the evolution of plant and fungi, is a rare event in most vertebrate lineages indicating differences in the capacity to adapt to genome duplications.

Here, we used the set of genes that were conserved at orthologous positions between five grass genomes and corrected from gene redundancy resulting from ancestral- and lineage-specific duplications to estimate the minimal ancestral genome gene number. We proposed that the cereals derive from a 33.6 Mb ancestor structured in five proto-chromosomes containing 9,138 proto-genes, a similar estimate to the 9,731 ancestral eudicot (11) and 11,502 ancestral angiosperm (17) gene repertoires. The 33.6-Mb ancestral genome

Fig. 3. Angiosperm evolutionary models. (A) Schematic representation of the monocot (Right) and eudicot (Left) evolutionary scenarios. The monocot chromosomes are represented with a five-color code to illuminate the evolution of segments from a common ancestor with five proto-chromosomes (named according to the rice nomenclature). The four shuffling events that have shaped the structure of the different grass genomes during their evolution from the common ancestor are indicated as α (whole genome duplication), γ (ancestral chromosome translocations and fusions), δ (family specific shuffling), and α (lineage specific shuffling). The seven eudicots proto-chromosomes are represented with different colors. The two scenarios proposed by Jaillon et al. (10) and Velasco et al. (11) are shown in red and green boxes, respectively. The different shuffling events that have shaped the structure of the A. thaliana, poplar and grape genomes during their evolution from a common ancestor (family specific shuffling), and lineage specific shuffling events depend on the model. The current structure of the genomes with the representation of the remaining ancestral duplicated blocks is represented at the bottom. (B) Monocot/Eudicot ancestral genomes comparison. The five monocot proto-chromosomes (with the associated 22 chromosome blocks) are depicted as vertical bars. The number (bars) and origin (seven colors) of orthologs identified between the 9,138 monocot and 9,731 eudicot genes is indicated for each of the 10 monocot proto-chromosome arms.

α, β events

1 WGD

Eudicots

Monocots

130–240 MYA

flowering plant Ancestor

20–40 MYA

Paleo-tetraploid

Paleo-hexaploid

29 MYA

Polkae

Intermediate n=10

Panicoideae

Intermediate n=10

5 MYA

α event

24 MYA

Cent5

A5S2

A5S3

A5L2

A5L1

Poplar

Grape

Rice

Triticeae

Maize

Sorghum

Paleo-tetraploid

5 MYA

Intermediate n=10

Cent11

A11S1

A11S2

A11S3

γ event

80–60 MYA

Paleo-tetraploid

90 MYA

9,138 monocot and 9,731 eudicot genes is indicated for each of the 10 monocot proto-chromosome arms.
ingly, these duplication-resistant genes have been conserved in duplication-resistant as they have been subjected particularly to ancestral grass genome gene set showed that 5 major GO classes are ontology (GO) as a reference, analysis of the evolution of the 9,138 the established monocot ancestor structure and using the rice gene identification of “duplication-sensitive” gene families (for which 1 contrast to animal genomes, it is not possible to identify synteny can be identified anymore. These results shed light on previous analyses of sympathy between Arabidopsis and rice where sympathy was detected only at the micro level for a few (<100) loci (34–36). The results obtained through comparisons between the endocyt and monocot proto-chromosomes demonstrate that, in contrast to animal genomes, it is not possible to identify synteny at the genome or chromosome levels across plant classes.

Evolutionary Fitness of the Proteogene Battery. Comparison of ancestral gene contents with those of current genomes permits the identification of “duplication-sensitive” gene families (for which 1 paralogous copy is lost in 1 genome compared with the others) and “duplication-resistant” gene families (for which paralogous copies are maintained leading to copy number amplification) (26). From the established monocot ancestor structure and using the rice gene ontology (GO) as a reference, analysis of the evolution of the 9,138 ancestral grass genome gene set showed that 5 major GO classes are duplication-resistant as they have been subjected particularly to duplications resulting in additional copies that potentially provided a selective advantage during evolution and adaptation. Interestingly, these duplication-resistant genes have been conserved in different genomes since the ancestral whole genome duplication δ event.

Our results for the monocot ancestor are consistent with the results obtained by Paterson and colleagues for the eudicots who showed that duplication-resistant gene families correspond to transcriptional regulators that are retained more significantly after WGD events (26, 32, 37). We recently demonstrated that within the 10 major paleo-duplications that cover 47.8% of the rice genome, only 12.6% of paralogous gene pairs are still conserved within sister blocks, leading to the conclusion that pseudogenization (loss of one copy) occurred for the vast majority of the paralogous pairs (57.4%) during 50–70 my of evolution (38). In contrast, genes that may not provide a selective advantage when duplicated would be restored rapidly to a singleton status. Thus, our data support previous findings in the eudicots and suggest preferential retention of duplicated genes involved in signal transduction, and transcription, in response to rapidly changing biotic and abiotic extrinsic factors compared with genes encoding products involved in relatively stable processes.

Additional sequences from other grass (i.e., Brachypodium) and non-cereal monocot genomes such as Musa acuminata (banana) or Ananas comosus (pineapple), along with sequences of basal eudicots such as Eschscholzia california (california poppy) or Papaver somniferum (opium poppy) and Aquilegia gigasformosa (columbine), and basal angiosperms such as Amborella trichopoda will further improve the accuracy of the paleogenomics studies in the major angiosperm clades and help to refine our model of plant genome evolution.

Materials and Methods

Details about the materials and methods used for the analysis regarding (i) nucleic acid sequence alignments, (ii) genome sequence databases, (iii) identification of duplicated and syntenic regions, (iv) graphical display, and (v) ancestor genome reconstruction can be found in SI Appendix. Enlarged resolution format of the figures is also available in SI Appendix.

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