Rapid amplification of four retrotransposon families promoted speciation and genome size expansion in the genus *Panax*

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Genome duplication and repeat multiplication contribute to genome evolution in plants. Our previous work identified a recent allotetraploidization event and five high-copy LTR retrotransposon (LTR-RT) families *PgDel*, *PgTat*, *PgAthila*, *PgTork*, and *PgOryco* in *Panax ginseng*. Here, using whole-genome sequences, we quantified major repeats in five *Panax* species and investigated their role in genome evolution. The diploids *P. japonicus*, *P. vietnemensis*, and *P. notoginseng* and the tetraploids *P. ginseng* and *P. quinquefolius* were analyzed alongside their relative *Aralia elata*. These species possess 0.8–4.9 Gb haploid genomes. The *PgDel*, *PgTat*, *PgAthila*, and *PgTork* LTR-RT superfamilies accounted for 39–52% of the *Panax* species genomes and 17% of the *A. elata* genome. *PgDel* included six subfamily members, each with a distinct genome distribution. In particular, the *PgDel1* subfamily occupied 23–35% of the *Panax* genomes and accounted for much of their genome size variation. *PgDel1* occupied 22.6% (0.8 Gb of 3.6 Gb) and 34.5% (1.7 Gb of 4.9 Gb) of the *P. ginseng* and *P. quinquefolius* genomes, respectively. Our findings indicate that the *P. quinquefolius* genome may have expanded due to rapid *PgDel1* amplification over the last million years as a result of environmental adaptation following migration from Asia to North America.

Nuclear genome sizes in flowering plants are diverse, and can vary over 2,400-fold, ranging from 63 Mb in *Genlisea margaretae* to 149 Gb in *Paris japonica*. This dramatic genome size variation is attributed to both whole-genome duplication and accumulation of repeated sequences, or repeats. During the diploidization process following genome duplication, euchromatic DNA is usually reduced by deletion of unnecessary paralogous regions, while heterochromatic DNA is often expanded by species-specific multiplication of repeats. Repeats are categorized into two major types: tandem repeats (TRs) and transposable elements (TEs). TRs exist in a head-to-tail arrangement in distinct chromosomal regions, generally found at centromeric, subtelomeric, and telomeric regions. By contrast, TEs are dispersed throughout the genome. TEs are classified based on their transposition mechanisms as class I (copy-and-paste) or class II (cut-and-paste). Class I TEs include the class I.1 LTR-retrotransposons (LTR-RTs) and the class I.2 non-LTR retrotransposons, whereas class II TEs include DNA transposons. Repetes play important roles in gene regulation, evolution, and adaptation. The family Araliaceae is composed of approximately 55 genera and 1,500 species, which include many valuable medicinal and ornamental plants. Within this family, the genus *Panax* contains economically important medicinal plants including the diploids *P. japonicus*, *P. vietnemensis*, and *P. notoginseng* (*2n = 2x = 24*), and the tetraploids *P. quinquefolius* and *P. ginseng* (*2n = 4x = 48*). These five species are perennial and absolute shade plants that have been used for medicinal purposes in Asia and North America because of their beneficial effects on human health. Although *Panax* species display relatively limited morphological diversity, their genome sizes...
Among these, Gypsy species, whereas in possessed a similar R-GP in the five elements, with a R-GP of 34.5% (Fig. 1). Our comparative analysis sequences and quantified each of these LTR-RTs in the genomes of five Panax genus. We also characterized the major repeats that occupied more than 35% of major repeats in the evolution of the Panax. These 13 repeats are high-copy, major repeats and are estimated to occupy more than ginseng Table 1. Whole genome sequence (WGS)-based quantification of major repeats in Panax genus, and Fig. S1). The reproducibility of R-GP estimation for each of major repeats was evaluated in each WGS. We then compared repeat quantification in WGS data sets with different genome coverages (0.00005–10x), as well as in WGS data sets from different libraries using (Supplementary Table S1). These 13 repeats are high-copy, major repeats and are estimated to occupy more than 35% of major repeats in the WGS datasets of five Panax species. We determined the amount of each repeat by calculating its genomic proportion (GP) in each WGS, via quantification of homologous nucleotides in each WGS based on repeat masking using RepeatMasker. We validated RepeatMasker-based GP (R-GP) estimation and the quantification of each major repeat using various WGS data sets. We then compared repeat quantification in WGS data sets with different genome coverages (0.00005–10x), as well as in WGS data sets from different libraries using P. ginseng cv. Chunpoong and in WGS data sets from different ginseng cultivars (Table 1, Supplementary Table S2, S4, and Fig. S1). The reproducibility of R-GP estimation for each of major repeats was evaluated in each WGS.

The R-GP of each repeat displayed little variation in datasets of the same WGS that represented nine different genome coverages, and low variation in datasets from four different WGS libraries created using the same ginseng cultivar (Table 1, Supplementary Table S2 and S4). The R-GP of PgDel1 was 23–26% among 11 cultivars (Supplementary Table S2 and Fig. S1).

Genomic quantification of major repeats in five Panax species. We used the above WGS-based R-GP estimation to quantify the major repeats in the genomes of five Panax species alongside 8 species from a related genus (Table 2). The quantification of repeats using PE reads corresponding to 0.3–1.5x haploid genome equivalents for each species revealed a R-GP of 46%, 45%, 50%, 41%, 53%, and 17% in P. japonicus, P. vietnamensis, P. notoginseng, P. ginseng, P. quinquefolius, and Aralia elata, respectively (Fig. 1). Each individual major repeat possessed a similar R-GP in the five Panax species, whereas in A. elata, the R-GP was comparatively low. The Ty3/Gypsy-type LTR-RT families, such as PgDel1–6, PgTat1 and 2, PgAthila, PgTork, and PgOryco, and two tandem repeat sequences, namely Pg167TR and 45 S rDNA (Supplementary Table S1). These 13 repeats are high-copy, major repeats and are estimated to occupy more than 41% of the P. ginseng genome (Supplementary Table S2, S3, and Fig. S1). Here, we aimed to quantify these major repeats in the WGS datasets of five Panax species. We determined the amount of each repeat by calculating its genomic proportion (GP) in each WGS, via quantification of homologous nucleotides in each WGS based on repeat masking using RepeatMasker. We validated RepeatMasker-based GP (R-GP) estimation and the quantification of each major repeat using various WGS data sets. We then compared repeat quantification in WGS data sets with different genome coverages (0.00005–10x), as well as in WGS data sets from different libraries using P. ginseng cv. Chunpoong and in WGS data sets from different ginseng cultivars (Table 1, Supplementary Table S2, S4, and Fig. S1).

| Amount of WGS (Mbp) | 0.18 | 0.36 | 3.6 | 36 | 1,800 | 3,600 | 18,000 | 36,000 |
|---------------------|------|------|-----|----|-------|-------|--------|--------|
| Genome coverage     | 0.00005x | 0.001x | 0.01x | 0.1x | 0.5x | 1x | 5x | 10x |
| PgDel1              | 21.82 | 23.79 | 24.1 | 23.75 | 24.11 | 21.9 | 24.23 | 24.09 | 24.06 |
| PgDel2              | 2.09  | 2.27  | 2.51 | 2.71  | 2.62  | 2.45 | 2.64  | 2.65  | 2.65  |
| PgDel3              | 3.17  | 2.84  | 2.51 | 2.52  | 2.61  | 2.53 | 2.59  | 2.6   | 4.04  |
| PgTat1              | 9.93  | 6.36  | 5.92 | 6.05  | 5.89  | 6.56 | 6.05  | 6.04  | 6.03  |
| PgTat2              | 0.54  | 0.64  | 0.64 | 0.7   | 0.7   | 0.9  | 0.72  | 0.72  | 11.29 |
| PgAthila            | 0.54  | 1.34  | 1.43 | 1.32  | 1.44  | 1.47 | 1.45  | 1.43  | 3.80  |
| PgTork              | 0.51  | 1.24  | 1.14 | 1.29  | 1.24  | 0.97 | 1.23  | 1.21  | 1.22  |
| PgOryco             | 0.07  | 0.09  | 0.1  | 0.11  | 0.09  | 0.1  | 0.1   | 0.1   | 13.53 |
| PgTR                | 1.57  | 1.18  | 1.07 | 1.11  | 1.2   | 2.06 | 1.16  | 1.19  | 1.21  |
| 45 S rDNA           | 0.54  | 0.73  | 0.7  | 0.76  | 0.69  | 0.72 | 0.75  | 0.75  | 51.11 |
| Total               | 40.71 | 40.47 | 40.09 | 40.24 | 40.68 | 40.92 | 40.85 | 40.79 | 40.77 | 0.75 |

Table 1. Summary of GP calculation for major repeats in WGS data sets with various genome coverage of P. ginseng cv. Chunpoong. CV: coefficient of variation. CV values were calculated for the GP values using 0.0001x-10x genome coverage WGS, except the GP for 0.00005x.

Results

Whole genome sequence (WGS)-based quantification of major repeats in P. ginseng. In P. ginseng, we recently reported 11 LTR-RT subfamilies contained within five superfamilies, namely PgDel1–6, PgTat1 and 2, PgAthila, PgTork, and PgOryco, and two tandem repeat sequences, namely Pg167TR and 45 S rDNA. We also characterized the major repeats that occupied more than 35% of the P. ginseng genome, namely five high-copy LTR-RT families. In this study, we aimed to explore the role of major repeats in the evolution of the Panax genus, which shows large genome size variation. Accordingly, we established a reliable quantification method for major repeats within a genome using low-coverage whole-genome sequences and quantified each of these LTR-RTs in the genomes of five Panax species. Our comparative analysis revealed dynamic impacts of these major repeats on genome size variation, speciation, and evolution in the Panax genus.
Species | Chromosome number | Genome size (Gb) | NGS sequencing platform | Average Read length (bp) | Reads (M)^d | Total bases (Mb)^e | Genome Coverage (%) | NABIC accession number
--- | --- | --- | --- | --- | --- | --- | --- | ---
P. ginseng | 2n = 48 | 3.6 | HiSeq | 101 | 36.2 | 3,605 | 1.00 | NN-0076-000001
P. quinquefolius | 2n = 48 | 4.9 | HiSeq | 101 | 12.4 | 1,236 | 0.25 | NN-0189-000001
P. notoginseng | 2n = 24 | 2.5 | MiSeq | 300 | 8.2 | 2,247 | 0.90 | NN-1913-000001
P. japonicus | 2n = 24 | ~2.0^f | MiSeq | 300 | 8.3 | 2,271 | 1.14 | NN-1914-000001
P. vietnamensis | 2n = 24 | 2.0 | NextSeq | 150 | 35.2 | 5,126 | 2.56 | NN-1915-000001
A. elata | 2n = 24^g | 0.8^h | HiSeq | 101 | 40.4 | 4,052 | 2.50 | NN-0919-000001

Table 2. Summary of WGS data of five Panax species and the related A. elata used for a survey of major repeats. aGenome size was estimated in the present study. bChromosome number was determined by DAPI (4′,6-diamidino-2-phenylindole) staining (Supplementary Fig. S2). cThe genome size of A. elata was considered to be approximately 0.8 Gb in this study, based on the genome sizes of related species. d,eQuality-controlled WGS reads were used in the current study.

Figure 1. Genomic proportion of the major repeats in Panax species and a related species. Genomic proportion (GP) of 13 repeats in five Panax species and the related species A. elata.

PgDel5 was more abundant in P. notoginseng and A. elata compared to that in other species. PgDel6 had 4.3% and 5% R-GP in the two diploids P. japonicus and P. vietnamensis, respectively, whereas it had 1.5–2.4% R-GP in the remaining three Panax species. The R-GP of PgTork varied dynamically between Panax species (Fig. 1).

Dynamics of the PgDel1 subfamily members in Panax species. We analyzed the structural dynamics of PgDel1 subfamily members in the Panax species. Five PgDel1 subfamily members (PgDel1_1–5) were identified from three complete BAC clone sequences (GenBank accession nos. KF357943, KF357944, and KF357942). These five members displayed relatively complete structures including both LTRs and an inner sequence, although there were nested insertions caused by other repeats or subsequent deletion events. Inspection of the complete unit of these repeats, which was 7.7–10.1 kb, revealed an overall similarity in the large structural variations in the LTR regions. To estimate the distribution of PgDel1 members in the P. ginseng genome, we mapped the 1x genome coverage Chunpoong WGS data onto the representative PgDel1_1 element because of the well-conserved LTR domains of PgDel1. Mapping depth had a range of 111–157,407 with an average of 50,952 (mode and median values were 48,399 and 47,503, respectively) (Fig. 2).

Cyto genomic mapping of PgDel1 and PgDel2 in three Panax species. To validate the R-GP variation identified via in silico analysis, we analyzed the distribution patterns of PgDel1 and PgDel2 by fluorescence in situ hybridization (FISH) using somatic metaphase chromosomes of three Panax species: P. notoginseng, as a representative of the three diploid Panax species, and the two tetraploids P. ginseng and P. quinquefolius. The PgDel1 elements displayed high-density FISH signals throughout the chromosomes in all three Panax species (Fig. 3A,B and C). The intensive FISH signal of PgDel1 throughout the chromosome regardless of the ploidy level of the species it originated from supported our in silico analysis results, which estimated 23–35% R-GP for PgDel1 in the Panax species (Figs 1 and 3A–C).

PgDel2 had nearly two-fold greater R-GP values in the three diploid Panax species compared to the two tetraploids (Fig. 1). Consistent with this result, FISH analysis revealed different distribution patterns of PgDel2 in diploid and tetraploid Panax species. PgDel2 signal was localized to pericentromeric regions in all 24 chromosomes of the diploid P. notoginseng, whereas strong PgDel2 signal was detected in half of the 48 chromosomes of both tetraploid Panax species (Fig. 3D,E and F). In these tetraploids, PgDel2 distribution was concentrated to the
pericentromeric regions in *P. ginseng* chromosomes but was more broadly located in *P. quinquefolius* chromosomes (Fig. 3E and F).

**Figure 2.** Structural characteristic of five *PgDel1* subfamily members. (A) Representation of the distribution of 1x WGS data of *P. ginseng* cv. CP. (B) Horizontal schematic diagrams of *PgDel1* subfamily members 1–5. Boxed orange triangles indicate LTR regions of *PgDel1*. Yellow boxes indicate the internal LTR-RTs domains of *PgDel1* detected in each subfamily member. (AP: aspartic protease, CH: chromodomain, GAG: capsid protein, INT: integrase, RH: RNase H, RT: reverse-transcriptase, and Zn: zinc knuckle). Homologous sequence were indicated as grey panels.

**Figure 3.** Fluorescence in situ hybridization (FISH) analysis of *PgDel1* and *PgDel2* distribution in *P. ginseng*, *P. quinquefolius*, and *P. notoginseng* chromosomes. The *PgDel1* FISH signals in somatic metaphase chromosomes of (A) *P. notoginseng* (purple), (B) *P. ginseng*, and (C) *P. quinquefolius*. The *PgDel2* FISH signals in somatic metaphase chromosomes of (D) *P. notoginseng*, (E) *P. ginseng* (blue), and (F) *P. quinquefolius* (blue). Bar = 10 μm.

**Contribution of major repeats to genome size variation.** We investigated the contribution of the four most abundant LTR-RT families, *PgDel*, *PgAthila*, *PgTat*, and *PgTork*, to the overall genome contents. Each family was present in varied proportions in the six analyzed species (Figs 1 and 4). Combined, the four LTR-RTs had a 39–52% R-GP in each of five species, corresponding to 0.9–2.6 Gb (Fig. 4). Of these repeats, *PgDel* occupied 30–41% of R-GP, accounting for 0.7–2.0 Gb. *PgTat* had a 5–7% R-GP, corresponding to 97–285 Mb. The estimated quantity of *PgTork* was 241 Mb in *P. quinquefolius*, whereas it was 24–57 Mb in the other four *Panax*
species. Interestingly, PgTork was the most abundant LTR-RT in the A. elata genome, occupying 7% R-GP (56 Mb) (Fig. 4).

Discussion
In this work, we used low-coverage WGS sequences to calculate the GP of major repeats. We estimated the prevalence of each repeat by determining GP using various WGS data sets, based on the calculation of masked homologous sequence in WGS reads by RepeatMasker

GP can also be calculated using clustered WGS reads or mapped WGS reads. Mapping-based GP (M-GP) and clustering-based GP (C-GP) calculations are based on numbers of homologous WGS reads, whereas R-GP calculation is based on real amounts of homologous sequences in WGS reads. We compared the ability of R-GP and M-GP methods to estimate PgDel1 GP using different WGS sets, which resulted in a consistent pattern whereby R-GP calculations estimated 3–4% more GP than M-GP calculations (Supplementary Fig. S3 and S4). This variation may be attributed to the difference in how homologous sequences are counted in both methods, namely the number of homologous reads and the number of homologous nucleotides for M-GP and R-GP, respectively.

The estimated GPs were highly reproducible for the major high-copy LTR-RTs, although we observed relatively high CV values (25–65%) for GP estimation of tandem repeats using different genome coverage and different WGS libraries (Table 1 and Supplementary Table S4). The number of tandem repeat reads might be uneven because of biased fragmentation during WGS library construction (Table 1 and Supplementary Table S4). Overall, though, the coefficients of variation (CVs) of the high copy PgDeII and the low copy PgOryco were 3.23% and 13.53%, respectively, when we estimated GP using various levels of genome coverage in the data sets, i.e., 0.0001–10x genome coverage for WGS reads of P. ginseng (Table 1). We observed slightly increased variation when we reduced the genome coverage below 0.0001x, but all the data showed similarly low CVs when we utilized over 0.0001x coverage WGS reads. As WGS data can be produced at low cost by high-throughput NGS processes and over 1 Mbp of WGS reads produced reproducible GP estimation, we conclude that genome coverage in WGS data is not a critical constraint limiting the application of this approach for analysis.

Although there is some variation, the GPs calculated here for the major repeats were reproducible and are thus representative of the abundance of each repeat in the genomes of the different species. However, it is possible that the true GP for each major repeat is higher than the GP estimate presented here because, in our analysis, only a single representative structure was used for each repeat and other structural variations were not considered. For example, five PgDeII elements displayed large structural variation in the LTR region and a large bias in WGS read mapping for the representative PgDeII family member in the P. ginseng genome (Fig. 2).

Our results point to tetraploidization and four LTR-RTs as the primary reasons for genome size variation in the genus Panax. Divergence of a common ancestor into the genera Panax and Aralia is predicted to have...
occurred approximately eight MYA. A. elata was estimated to have an approximate haploid genome equivalent size of 0.8 Gb on 12 chromosome pairs (Supplementary Fig. S2). However, the genome sizes of Panax species (2.0–4.9 Gb) are much larger than that of A. elata. We propose that the multiplication of some major repeats influenced the genome size in the Panax lineage. In particular, a large proportion of the increased genome size can be explained by multiplication of the four LTR-RTs investigated here, which occupied 0.9–2.6 Gb in the Panax lineage (Fig. 4). The GP of the four LTR-RTs was 39% (1.4 Gb) and 52% (2.6 Gb) in two tetraploids, P. ginseng and P. quinquefolius, respectively, and 44–49% (0.9–1.2 Gb) in the three diploid Panax species. Among them, PgDel was the predominant repeat with a GP of 30–41%, which corresponds to 0.7–2.0 Gb in the five Panax species.

LTR-RTs make up a large proportion of the genomes of many higher plants. The repeats can play an important role as promoters of genomic diversification and speciation. It is possible that, even in the same genus, a rapid burst of retrotransposition can induce genome size variance with different evolutionary effects, as observed for Oryza, Nicotiana, and Genlisea. Here we investigated abundant, high-copy LTR-RTs and performed a comparative analysis of these repeats in Panax species to understand their influence on genome evolution. The presence of these repeats in five Panax species and a further related species suggests that they likely existed in the genome of a common ancestor. However, extensive multiplication of LTR-RTs occurred only in the Panax genus and appears to have a decisive effect on the expansion of the genome sizes in Panax species (Figs 1 and 4). This finding suggests that the repeat amplification occurred concomitantly with or following divergence in the five Panax species during the last eight million years.

We identified six PgDel subfamilies based on LTR sequences from P. ginseng BAC clone sequences. Among them, PgDel1 was highly abundant in each Panax species. The abundance, sequence diversity, and cytogenetic distribution of PgDel1 LTR-RTs indicated that considerable multiplication and transposition may have occurred across the five Panax species genomes (Figs 1, 2, 3 and 4). We found a positive correlation (coefficient of 0.6 with a p-value of 0.40) between the B-GP values for PgDel1 and the genome size of each Panax species (Table 2 and Fig. 4). This correlation indicates that the accumulation of PgDel1 elements has greatly contributed to the increased genome sizes in the genus Panax. In this regard, we speculate that the genome size of P. japonicus might be below 2.0 Gb, on the relatively small PgDel1 GP we found in the diploid Panax species (Figs 1 and 4).

Correlation between PgDel1 abundance and genome size in Panax species could explain the expansive genome of P. quinquefolius, which is the largest within the Panax genus. The two tetraploids P. ginseng and P. quinquefolius were reported to exhibit a difference of 1.3 Gb. Based on divergence of orthologous gene pairs, we estimated that these species diverged approximately one MYA, following the recent allotetraploidization two MYA. The considerable disparity in genome size that has evolved between P. ginseng and P. quinquefolius is largely explained by the different amount of PgDel1 in each genome, which is 0.8 and 1.7 Gb, respectively, indicating that PgDel1 was exclusively amplified in P. quinquefolius during last one MYA.

The difference between PgDel1 GP in P. ginseng and P. quinquefolius can be explained by two hypotheses concerning TE dynamics. The first hypothesis is that there was a considerable loss of PgDel1 GP in P. ginseng after speciation. Polyploidization often results in genome downsizing via expulsion of genomic DNA, mostly repetitive DNA sequences, for stable meiotic rebuilding in nascent polyploids. The second hypothesis is that there was a sizeable expansion of PgDel1 GP in P. quinquefolius after speciation. We believe that the second hypothesis holds more merit than the first. Drastic environmental change could have triggered epigenetic restructuring, resulting in the unusual accumulation of LTR-RTs in P. quinquefolius. P. quinquefolius is said to have migrated from Asia to America through the Bering land bridge during glacial and interglacial cycles one MYA. Consequently, P. quinquefolius would have been exposed to extreme abiotic stress during the process of migration and adaptation to new habitats. The influence of PgDel1 amplification in genome organization and gene function accordingly might play an important role in the interspecific genomic barriers between species.

PgDel1 made up a large proportion of the genome in all five Panax species analyzed in this study. In addition, other PgDel subfamily members also had notable genome distributions in the Panax species. PgDel2 occupied approximately 1.4% GP in the two tetraploids and 2.8% GP in the three diploid Panax species (Fig. 1). This variation in PgDel2 GP between diploids and tetraploids was confirmed by cytogenetic analysis using FISH. In the tetraploids P. ginseng and P. quinquefolius, PgDel2 signals were observed in half of the chromosomes whereas all chromosomes of the diploid P. notoginseng displayed PgDel2 signals (Fig. 3E, and F). PgDel5 and PgDel6 showed large differences among three Panax species. P. notoginseng had approximately twice the amount of PgDel5 than the other Panax species, and P. japonicus and P. vietnamensis had more abundant PgDel6 compared to the other species. These findings highlight the likely importance of the PgDel subfamily contribution to diversification of Panax species.

**Materials and Methods**

**Plant materials, genomic DNA isolation, and Illumina sequencing.** Eleven P. ginseng cultivars as well as P. quinquefolius, P. notoginseng, P. japonicus, P. vietnamensis and A. elata were used for genomic DNA preparation and sequencing (Table 2 and Supplementary Table S3). P. ginseng cv. Chumpoon was used as a representative for GP estimation in the current analysis. Leaf tissue for the above species, apart for P. notoginseng, P. japonicus, P. vietnamensis, and A. elata, was obtained from the ginseng farms of Seoul National University and Korean Ginseng Corporation (http://www.kgc.or.kr). A. elata and P. vietnamensis leaf tissue was collected from Susinogapy Corporation (http://www.susinogamy.com), Korea, and Da Lat City, Tay Nguyen Institute of Scientific Research, Vietnam, respectively. P. notoginseng and P. japonicus leaf tissue was collected from Dafang Country, Guizhou province, and Enshi County, Hubei province, China, respectively.

Genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method. All genomic libraries were prepared according to the recommended Illumina paired-end standard protocol (http://www.illumina.com). The whole genomes of those plants listed in Table 2 and Supplementary Table S3 were sequenced using an Illumina genome analyzer at the National Instrumentation Center for Environmental...
Major repeat sequences of Panax ginseng. In our previous study, we described the major repeats of P. ginseng including 11 LTR-RTs and two tandem repeat sequences (Pg167TR and 45 S rDNA), which occupy more than one third of the genome. These reference sequences were used as queries to estimate their abundance in Panax and Aralia genomes. Most of LTR-RTs of major repeats analyzed in this work have a complete structure that includes both flanking LTRs and inter-LTR domains, with the exception of PgAthila that has one LTR. The 45 S rDNA of P. ginseng was used as a representative rDNA sequence for all Panax species (Supplementary Table S1 and Data S1).

Quantification of major repeats using WGS. The GP of each repeat was quantified by masking nucleotides of WGS reads into the representative repeat sequence using RepeatMasker (ver. 4.0.6). WGS reads were trimmed based on their quality score (minimum quality score: \( \geq 20 \)) using the software Trimmomatic ver. 0.33. WGS reads were directly surveyed for homology to each repeat using RepeatMasker, using the slow search parameters and option that does not mask low complexity DNA or simple repeats (applying ‘-s –no low’). Homologous nucleotides were masked and the amounts of masked nucleotides were counted to calculate GP for each repeat. The RepeatMasker-based genomic proportion (R-GP) was calculated as the proportion that masked nucleotides make of total nucleotides in each data set: R-GP (\%) = (masked read length/total read length) \( \times \) 100. The actual amounts of each repeat in the genome was estimated based on the R-GP and the size of the genome: Repeat amount = (R-GP/100) \( \times \) genome size (Fig. 4B). The mapping-based GP (M-GP) and copy number of PgDel1_1 LTR-RTs were estimated using CLC mapper ver. 4.21.104315 (CLC Inc, Aarhus, Denmark) with the parameters of minimum 50% fraction of the read and 80% similarity (Fig. 2A, Supplementary Fig. S3 and S4).

Fluorescence in situ hybridization (FISH) analysis. Preparation of P. notoginseng, P. ginseng, and P. quinquefolius chromosome spreads and FISH procedures were performed according to dual-color FISH analysis protocols.

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Author Contributions

J.L. and T.J.Y. conceived and designed the experiments. J.L., N.E.W., V.B.N., W.J. and N.H.K. prepared the samples and performed the experiments. J.L., N.E.W., H.I.C. and T.J.Y. analyzed the data. J.L., N.E.W., S.P., S.C.L., L.G. and T.J.Y. wrote the manuscript.

Additional Information

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