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Characterization of the Complete Chloroplast Genomes of Sequences of Two Diploid Species: *Paeonia lactiflora* 'Da Fugui' and *Paeonia ostii* 'Fengdan' in the Paeoniaceae Family

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Abstract

Paeonia ostii 'Fengdan' and *Paeonia lactiflora* 'Da Fugui' are two species of the same genus that are closely related. *Paeonia ostii* 'Fengdan' has a high nutritional and health value in woody oil crops, and is also a good parent material for intraspecific and interspecific hybridization; *Paeonia lactiflora* 'Da Fugui' is a kind of peony variety with high ornamental value. Both 'Fengdan' and 'Da Fugui'are diploid species. The complete cp genome sizes of *Paeonia ostii* 'Fengdan' and *Paeonia lactiflora* 'Da Fugui' were 152731 and 157372bp, respectively. *Paeonia ostii* 'Fengdan' Small Single Copy Area (SSC) is 16968 bp, the Large Single Copy Area (LSC) is 84402 bp, the Inverse Repeat region (IR) is 25681 bp. *Paeonia ostii* 'Fengdan' encods a total of 151 genes, including 101 protein-coding genes, 43 tRNA genes and 7 rRNA genes. In addition, the *Paeonia lactiflora* 'Da Fugui' Small Single Copy Area (SSC) is 14822 bp, the Large Single Copy Area (LSC) is 82,043 bp, the Ieverse Repeat region (IR) is 30254 bp. *Paeonia lactiflora* 'Da Fugui' encods a total of 165 genes, including 110 protein-coding genes, 47 tRNA genes and 8 rRNA genes. The cluster analysis of the NJ(Neighbor-joining) tree showed that Paeoniae genus is distributed in a large branch, and is closely related to the other three genera. *Paeonia ostii* 'Fengdan' and *Paeonia sp.* Sd0052 are clustered together with herbaceous peony, while *Paeonia lactiflora* 'Da Fugui' is clustered more closely to peony.

Keywords: *Paeonia ostii* 'Fengdan'; *Paeonia lactiflora* 'Da Fugui'; Chloroplast; Genome; NJ phylogenetic tree

Introduction

Paeonia ostii 'Fengdan' and *Paeonia lactiflora* 'Da Fugui' are two species of *paeoniaceae* family. *Paeonia ostii* 'Fengdan' belongs to the peony species. It is native to China; the quality of *Paeonia ostii* 'Fengdan' in Tongling County, Anhui Province is considered containing the highest medicinal value which is named Tongling peony. *Paeonia ostii* 'Fengdan' plants are tall and straight, with a large annual growth. The annual branches can reach 50 cm in length. They have large long leaves with long oval to long ovate lanceolate shapes. The flower is dominated by single flower with a few petals are slightly increased which show a lotus flower shape. The flowers are mostly white, and few of them show different colors such as purple red and pink. They are called "Feng Dan White", "Feng Dan Purple" and "Feng Dan Pink". Because the flower pattern is single and the color is monotonous, it is rarely used in ornamental gardening, mainly used as the medicinal plant, and its root can be processed into the famous Chinese herbal medicine root bark of *Paeonia suffruticosa* [1,2]. *Paeonia ostii* 'Fengdan' have high seed setting rate and full seeds. Recent studies proved that its seeds contain rich unsaturated fatty acids, such as alpha linolenic acid, linoleic acid and oleic acid [3-5], and has a positive effect on human health [6]. *Paeonia lactiflora* 'Da Fugui' belongs to the *Paeonia lactiflora*, which is a traditional variety. Its plant shape is very short, the color is purple red, and the flower is rose or rose garret type. The number of petals decreases from the outer layer to the inner layer. It has more side buds than tree peony and is easy to form flowers and the shape of *Paeonia lactiflora* 'Da Fugui' like a large bouquet in full bloom. It is an early flower variety, with strong growth of luxuriant stems and leaves. When blooming, the flowers are great quantity and neat with graceful and luxurious temperament. It is known that *Paeonia lactiflora* has strong medicinal properties. Its roots can be used as medicine and plays a significant role in regulating the immune system, cardiovascular system and central nervous system [7]. Both *Paeonia ostii* 'Fengdan' (2n =10) and *Paeonia lactiflora* 'Da Fugui' (2n =10) have ornamental and application value with karyotype formula: 2n =2x =10 =6m +2sm +2st, 2A type [8,9] two plants are comparable in the chloroplast genome level. The sequencing of the chloroplast genome of *Paeonia ostii* 'Fengdan' and *Paeonia lactiflora* 'Da Fugui' is completed and can more systematically analyze the evolution of the chloroplast genome of Paeoniaceae. It provides a valuable reference for further exploration of the application of genome rearrangement in phylogenetic construction.

Materials and Methods

Chloroplast genome assembly and annotation

In this study, we reported the complete chloroplast genome of *Paeonia ostii* 'Fengdan' and *Paeonia lactiflora* 'Da Fugui'. The fresh leaves of *Paeonia ostii* 'Fengdan' and *Paeonia lactiflora* 'Da Fugui' were collected from the peony and herbaceous peony germplasm resource garden of Yangzhou University, Jiangsu, Yangzhou, China (32°23′ N, 119°24′ E). Genomic DNA was sequenced using the Illumina Hiseq platform. Data filtering is performed on Raw Data, and the joint sequence and low quality Reads are removed to obtain high quality 7.09 Gb and 5.51Gb Clean Data. The chloroplast genome sequences of the reference species were assembled by Clean Data with NOVOPlasty [10] software, and the results of chloroplast assembly were obtained. The CpGAVAS [11] software was used to annotate the genomic structure of the chloroplast

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genome, to further determine the encoding sequence tRNA and rRNA, and in some cases, manually adjusted after the BLAST search. The annotation sequence is then logged into GenBank, and the accession number is SRR7614768 *Paeonia lactiflora*, SRR7614723 *Paeonia ostii*.

Genome comparison

MISA (MIcroSAtellite identification tool) is software which can identify Simple Sequence Repeat (SSR). SSR analysis of genomic sequences is using MISA software [12,13]. After alignment through MAFFT version 7 software [14], the sequence was manually adjusted with BioEdit, especially for the reversal site. We then performed microstructural mutation detection and sliding window analysis to evaluate the variability (Pi) of the entire plastomer in the DnaSP version 5 software [15]. The window length was set to 600bp and the step size is 200bp.

Phylogenetic analysis

To explore the relationship between *Paeonia ostii* 'Fengdan' and *Paeonia lactiflora* 'Da Fugui', we downloaded the other 16 species of chloroplast genome sequences from NCBI. The DOGMA [16] software (http://dogma.ccbb.utexas.edu/) was used to predict the encoding gene and the non coded RNA, in which the predicted identity threshold was set to 40, and the other parameters were the default values. By comparing the sequence of gene amino acid sequences of two species in *Paeonia ostii* 'Fengdan' and *Paeonia lactiflora* 'Da Fugui' species by MEGA5.0 software, we find out the genes with large difference, and select *matK*, *rps16*, *psaA*, *rbcL*, *accD*, *petA*, *rps8*, *rp116*, *ndhB*, *ndhF*, and *ycf1* 11 genes. The Neighbor-Joining phylogenetic tree was constructed using MEGA 5 software, and the number of repeated sampling was 1000.

Results

Chloroplast genome assembly and annotation

The full CP genome size of *Paeonia ostii* 'Fengdan' and *Paeonia lactiflora* 'Da Fugui' are 152731 and 157372bp respectively Figure 1. The small single copy area (SSC) of *Paeonia ostii* 'Fengdan' (SSC) is 16968bp, the large single copy area (LSC) is 84402bp, and the reverse repeat area (IR) is 25681bp. A total of 151 genes are encoded, including 101 protein encoding genes, 43 tRNA genes and 7 rRNA genes. In addition, the small single copy area (SSC) of *Paeonia lactiflora* 'Da Fugui' is 14822bp, the large single copy area (LSC) is 82043bp, and the reverse repeat area (IR) is 30254bp. A total of 165 genes are encoded, including 110 protein encoding genes, 47 tRNA genes and 8 rRNA genes (Table 1).

Analysis of SSR sequences

In chloroplast SSR analysis, diploid types are important for revealing the genetic diversity of population chloroplasts. *Paeonia ostii* 'Fengdan' has 2 complex repeat types SSR, 39 single-base repeat SSR, and 7 base repeat SSR; *Paeonia lactiflora* 'Da Fugui' has 5 complex repeat types SSR, 38 single-base repeat SSR and 5 two-bases repeat SSR (Table 2). We also list specific information about some types of SSRs (Table 3).

Indel and SNP between *Paeonia lactiflora* 'Da Fugui' and *Paeonia ostii* 'Fengdan'

In *Paeonia lactiflora* 'Da Fugui' and *Paeonia ostii* 'Fengdan' plastomes, we detected a lot of Indel and SNP. A total number of 5629 Indel, including 494 insert, 5135 delete. A total number of SNP is 998, including 593 two-transitions and 405 four-transversions. We show the quantitative relationship between transitions and transversions in Figure 2.

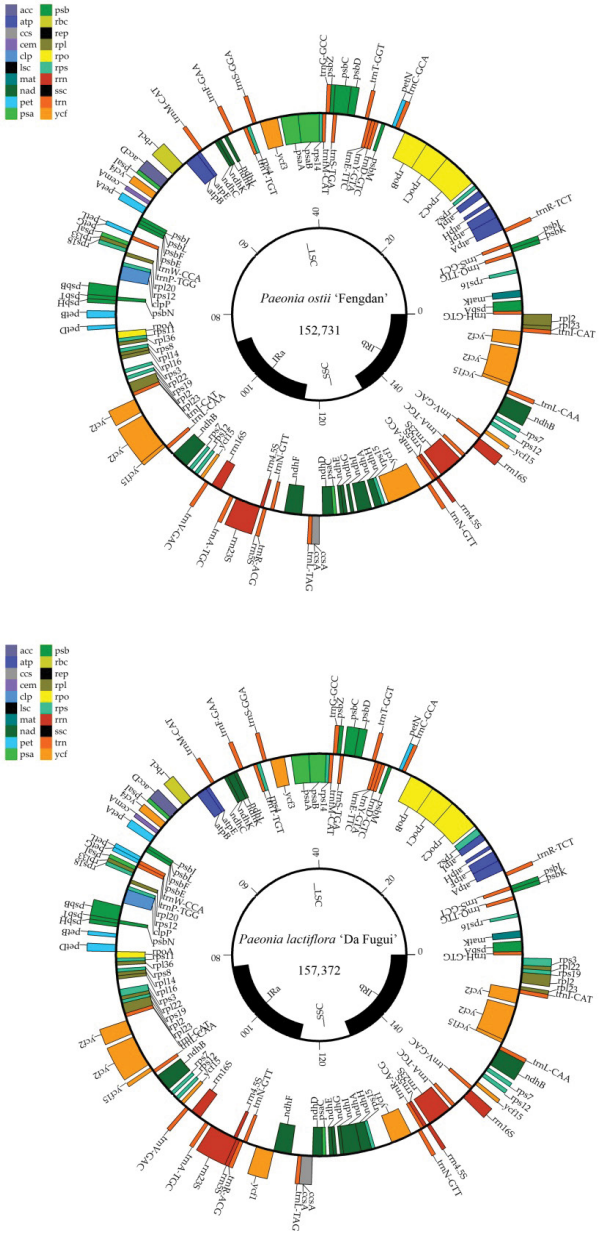

Figure 1: Physical map of the chloroplast genomes of *Paeonia ostii* 'Fengdan' and *Paeonia lactiflora* 'Da Fugui'.

	Paeonia ostii 'Fengdan'	*Paeonia lactiflora* 'Da Fugui'
Total cpDNA size	152731	157372
Length of large single copy (LSC) region	84402	82043
Length of inverted repeat (IRs) region	25681	30254
Length of small single copy (SSC) region	16968	14822
Total number of genes	151	165
Protein encoding	101	110
tRNA	43	47
rRNA	7	8

Table 1: Summary of two complete plastomes of *Paeonia*.

	Paeonia ostii 'Fengdan'	*Paeonia lactiflora* 'Da Fugui'
C (Complex repeat type)	2	5
P1 (Single base repeat)	39	38
P2 (Two base repeats)	7	5
Total	48	48

Table 2: Different types of SSR of 'Feng Dan' and 'Da Fugui'.

ID	Length	SSR_type	SSR	Start	End
Paeonia ostii 'Fengdan'	152731	p1	(A)11	282	292
Paeonia ostii 'Fengdan'	152731	p1	(T)11	2238	2248
Paeonia ostii 'Fengdan'	152731	p1	(T)10	3123	3132
Paeonia ostii 'Fengdan'	152731	p1	(A)10	4235	4244
Paeonia lactiflora 'Da Fugui'	157372	p1	(A)11	6385	6395
Paeonia lactiflora 'Da Fugui'	157372	p1	(T)10	6940	6949
Paeonia lactiflora 'Da Fugui'	157372	p2	(TA)7	10339	10352
Paeonia lactiflora 'Da Fugui'	157372	p1	(A)17	12054	12070

Table 3: SSR analysis result of 'Feng Dan' and 'Da Fugui'.

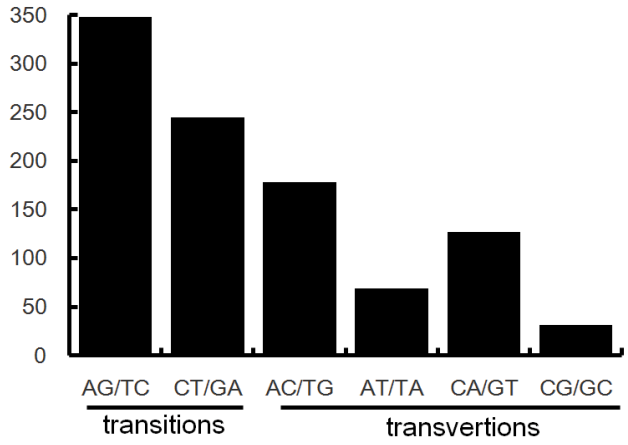

Figure 2: The patterns of nucleotide substitutions among the *Paeonia lactiflora* 'Da Fugui' and *Paeonia ostii* 'Fengdan'. The patterns were divided into six types as indicated by the six non-strand-specific base-substitution types. The plastome of *Paeonia lactiflora* 'Da Fugui' was used as a reference.

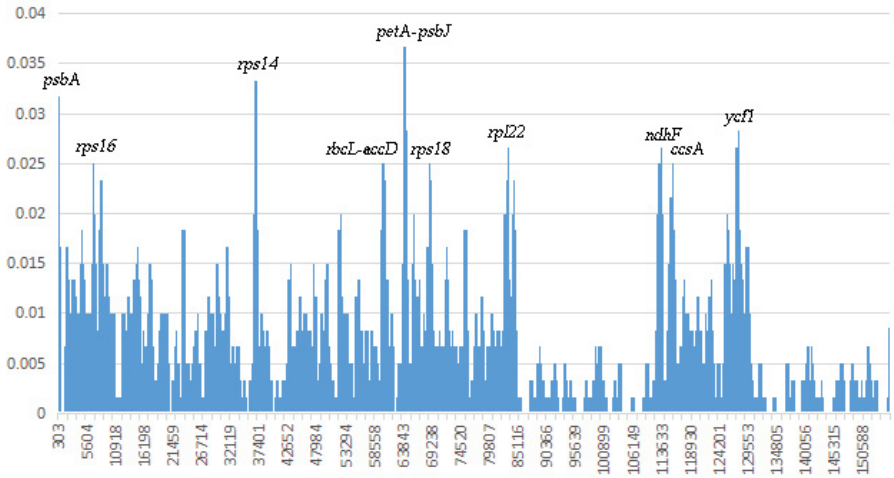

Figure 3: Sliding window analysis of the whole plastomes of *Paeonia lactiflora* 'Da Fugui' and *Paeonia ostii* 'Fengdan'. (Window length 600 bp, step size 200 bp).

Identification of the most variable regions in *Paeonia lactiflora* 'Da Fugui' and *Paeonia ostii* 'Fengdan'

SNP and indel markers are not randomly distributed in the genome but cluster in divergence hot spots. Between the *Paeonia lactiflora* 'Da Fugui' and *Paeonia ostii* 'Fengdan', these values varied from 0 to 0.0366, of which 10 are high variable region (Pi >0.025). It was including *psbA*, *rps16*, *rps14*, *rbcL-accD*, *petA-psbJ*, *rps18*, *rpl22*, *ndhF*, *ccsA* and 10 regions of *ycf1* were mapped (Figure 3). In these variable loci, *petA-psbJ* has the highest value (Pi >0.035); the values of *psbA* and *rps14* are much higher than the other 7 variable loci (Pi > 0.03). Among them, seven variable loci are located in the LSC region and are excellent candidate markers for phylogenetic analysis.

Phylogenetic analysis

The result of cluster analysis Figure 4 shows that the Paeonia genus is clustered in a large branch and is far from the other three genera. *Paeonia ostii* 'Fengdan' and *Paeonia* sp. Sd0052 are clustered together with herbaceous peony, while *Paeonia lactiflora* 'Da Fugui' is clustered with peony. The results of the clustering of *Paeonia ostii* 'Fengdan' and herbaceous peony provide a basis for the good hybridization between *Paeonia ostii* 'Fengdan' as a paternal and the herbaceous peony as a female parent, while the clustering of *Paeonia lactiflora* 'Da Fugui' and peony requires further research.

Discussion

This study reports two complete chloroplast genomes of *Paeonia lactiflora* 'Da Fugui' and *Paeonia ostii* 'Fengdan' (Figure 1), obtained using Illumina high-throughput sequencing technology. The two genomes are similar in structure but vary in size. *Paeonia ostii* 'Fengdan' is 4641 bp shorter than *Paeonia lactiflora* 'Da Fugui'. For a species identification and population structure analysis, rapidly developing molecular markers such as indels and SNPs have proven to have great potential.

All mutational kinetics, including SNPs and indels, create highly

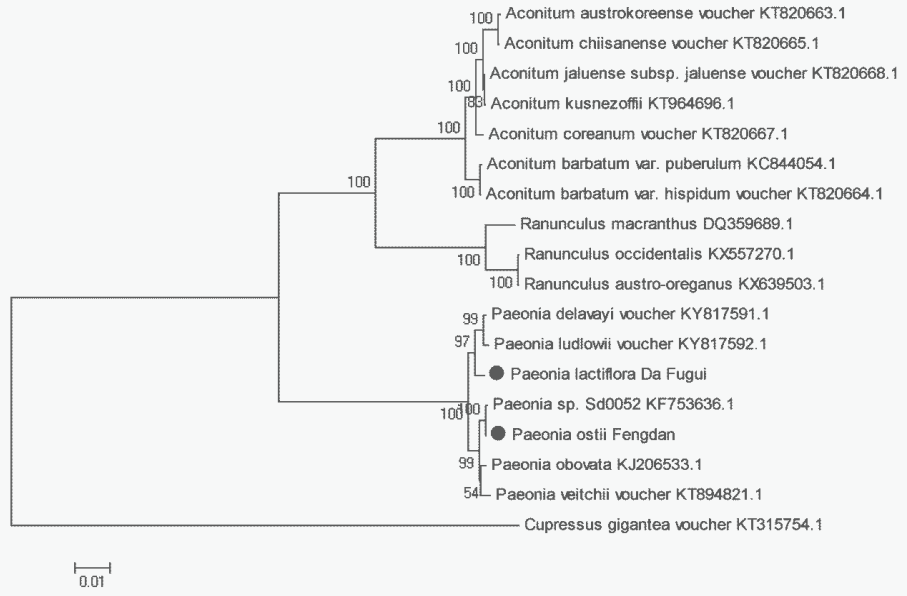

Figure 4: Phylogenetic relationships among 18 species based on the maximum parsimony analysis of all chloroplast protein-coding genes. The bootstrap values next to the branches are based on 1000 resamplings.

variable regions in the genome. We identified 10 highly variable regions psbA, rps16, rps14, rbcL-accD, petA-psbJ, rps18, rpl22, ndhF, ccsA and ycf1 in the chloroplast genome of *Paeonia ostii* 'Fengdan' and *Paeonia lactiflora* 'Da Fugui'. Dong [17] scanned the entire chloroplast genomes of 12 genera to search for highly variable regions, and then found the most variable (in order from highest variability to lowest) were intergenic regions *ycf1-a*, *trnK*, *rpl32-trnL*, and *trnH-psbA*, followed by *Trn*(SUGA)-*trnG*(UCC), *petA-psbJ*, *rps16-trnQ*, *ndhC-trnV*, *ycf1-b*, *ndhF*, *rpoB-trnC*, *psbE-petL*, and *rbcL-accD*. Three loci, *trnS* (UGA)-*trnG* (UCC), *trnT-psbD*, and *trnW-psaJ*. In the study of Song, seven highly variable loci between *M. yunnanensis* and *M. balansae* plastomes, were identified, including the second intron *clpP*, *ndhF-rpl32*, *trnQ-psbI*, *rps8-rpl14*, *ycf2*, *rpl32- trnL* and *ycf1* [18]. *Ycf1* is the most variable plastid genome region and can serve as a core barcode of land plants [19]. The altered regions were successfully used for phylogenetic studies of intergeneric and major branches.

At present, there are few analyses of the chloroplast genomes of the two diploid varieties of *Paeonia ostii* 'Fengdan'and *Paeonia lactiflora* 'Da Fugui'. And *Paeonia ostii* 'Fengdan' and *Paeonia lactiflora* 'Da Fugui' are all genus *Paeonia*, and one is an herb and the other is a woody one. After reviewing the literature, we found that Guohas done the *Paeonia ostii* chloroplast genome sequencing [20]. The gene length of its chloroplast is the shortest among the five combinations of *Paeonia delavayi, Paeonia ludlowii, Paeonia obovata, Paeonia veitchii* and *Paeonia ostii*. They speculate that the different lengths of the IR region are the main reason for the change in sequence length. After calculating the sequence identity between the five peony chloroplast DNAs, the results of the comparison showed that the IR (A/B) region was less divergent than the LSC and SSC regions. In SSR analysis, *Paeonia ostii* dinucleotide is the least common of the five. The 10 highly variable regions we have selected are better used for phylogenetic studies of *Paeonia* species. And we encourage researchers studying peony to use the 10 highly variable regions identified in this study for phylogenetic analysis.

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