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BRIEF COMMUNICATION

Different maternal genome donor to *Kengyilia* species inferred from chloroplast *trnL-F* sequences

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Abstract

To reveal the maternal donor of species in genus *Kengyilia*, the chloroplast *trnL-F* sequences of 14 *Kengyilia* species and several related diploid species were analyzed by using Maximum Parsimony (MP) and Bayesian Inference (BI) methods. The species in *Kengyilia* were clustered in different clades, which indicated that *Agropyron* (P) is the likely maternal genome donor to *Kengyilia melanthera*, *K. mutica* and *K. thoroldiana*, while the maternal donor to *Kengyilia batalinii*, *K. nana*, *K. kokonorica*, *K. kaschgarica*, *K. hirsuta*, *K. alatavica*, *K. gobicola*, *K. zhaosuensis*, *K. rigidula*, *K. longiglumis* and *K. grandiglumis* was St or Y *Roegneria* genome.

Additional key words: *Agropyron,* cluster analysis, phylogeny, *Roegneria,* StYP genomes, *Triticeae*.

Kengyilia Yen *et* J.L. Yang is a perennial genus in *Triticeae* (*Poaceae*), with about thirty species worldwide. Morphologically, species in *Kengyilia* are intermediate between species of *Roegneria* C. Koch and *Agropyron* Gaertn. (Yen and Yang 1990, Cai and Zhi 1999) Cytologically, these species are allohexaploid grasses with StYP genomes (Jensen 1990, 1996, Zhou 1994, Zhang *et al*. 2000), and the StY genomes of *Kengyilia* come from *Roegneria* and the P genome is derived from *Agropyron* (Yen and Yang 1990). Many molecular and cytological methods were used to study the phylogeny, genetic diversity and genomic constitutions of *Kengyilia* species (Zhang *et al*. 2000, 2005, Zeng *et al*. 2008). However, the maternal donor to these species is in dispute. Yen *et al*. (2006) indicated that the female parents of *Kengyilia* species was the species from *Agropyron* and their male parental species ought to have the St and Y haplomes based on the karyotype study on *Kengyilia* species. Nevertheless, according to the sequence data of the chloroplast *ndhF* gene, Redinbaugh *et al*. (2000) found a strong preference for cpDNA inheritance from the St nuclear genome-containing parent in hybridization between different *Triticeae* species. Liu *et al*. (2006) reported that *Pseudoroegneria* is the maternal genome donor to *Elymus* species based on *trnL-F* sequences.

The chloroplast genome is maternally inherited in grasses and provides a mechanism to determine the direction of hybridization in polyploid evolution (Jones *et al*. 2000). The chloroplast *trnL-F* sequence region includes the *trnL* (UAA) intron, *trnL* (UAA) 3'exon, *trnF* gene and an intergenic spacer between the *trnL* (UAA) 3'exon and *trnF* (GAA) gene (Taberlet *et al*. 1991) and is often used as a valuable source of markers for identifying the maternal donors of polyploides and for revealing phylogenetic relationships of related species (Mason-Gamer *et al*. 2002, Liu *et al*. 2006). In the present study, the *trnL-F* sequences from 30 accessions, including 14 species of *Kengyilia* and the diploid perennial species of seven basic genomes (St, P, E^e, E^b, W, H, Ns) were analyzed (Table 1). *Bromus catharticus* Vahl. was used as outgroup. The objectives are to identify the maternal genome donor of the *Kengyilia* species and to evaluate the phylogenetic relationships among these species. The accessions with PI numbers were kindly provided by American National Plant Germplasm System (Pullman, Washington, USA) and the others were collected from the

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Abbreviations: CTAB - cetyltrimethylammonium bromide; PCR - polymerase chain reaction.

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Total DNA was extracted from about 50 mg of fresh leaves from bulked 10 - 15 plants per accession using the CTAB method (Doyle and Doyle 1990). The chloroplast tRNA genes *trnL*-5', *trnL*-3' and *trnF*, along with their intervening noncoding regions, were amplified using the primers c (5'-CGAAATCGGTAGACGCTACG-3') and f (5'-ATTTGAACTGGTGACACGAG-3') designed by Taberlet *et al*. (1991). PCR amplification of the cpDNA was performed in a total reaction volumes of 0.025 cm^3 containing 1 × ExTaq polymerase buffer, 1.5 mM $MgCl_2$, 1 μM of each primer, 0.4 mM of dNTP mixture, 20 - 40 ng of template DNA, 1.25 U of ExTaq polymerase (*TaKaRa*, Dalian, Liaoning, China) and sterile water to the final volume. The PCR reactions were carried out in a *GeneAmp 9700* thermal cycler (*Applied Biosystems*, Foster City, USA). The thermal cycling profile consisted of an initial denaturation step at 95 °C for 5 min, 30 cycles

Table 1. The species of *Kengyilia* and its related diploid species used in this study (* - data from published sequences in the GenBank; http:// www.ncbi.nlm.nih.gov).

Species	Genome	Voucher	Locality	GenBank No.
Kengyilia Yen *et* Yang				
K. gobicola Yen and J.L. Yang	StYP	Y9502	Taxkorgan,Xinjiang, China	EF396986
K. alatavica (Drobov) J.L. Yang, Yen *et* Baum	StYP	PI565001	Kazakhstan	EU131903
K. batalinii (Krassn) J.L. Yang, Yen *et* Baum	StYP	PI565002	Kazakhstan	EU131904
K. grandiglumis (Keng *et* S.L. Chen) J.L. Yang, Yen *et* Baum	StYP	Y2857	Haiyan, Qinghai, China	EU131906
K. hirsuta (Keng *et* S.L. Chen) J.L. Yang, Yen *et* Baum	StYP	ZY3069	Hezuo, Gansu, China	EU131907
K. kaschgarica (D.F. Cui) L.B. Cai	StYP	Y9506	Taxkorgan, Xinjiang, China	EU131905
K. kokonorica (Keng *et* S.L. Chen) J.L. Yang, Yen *et* Baum	StYP	Y2880	Gonghe,Qinghai, China	EU131908
K. longiglumis (Keng *et* S. L. Chen) J. L. Yang, Yen *et* Baum	StYP	ZY3119	Xiahe, Gansu, China	EU131909
K. melanthera (Keng) J.L. Yang, Yen *et* Baum	StYP	ZY3146	Hongyuan, Sichuan, China	EF396987
K. mutica (Keng *et* S.L. Chen) J.L. Yang, Yen *et* Baum	StYP	Y2873	Geermu,Qinghai, China	EU131910
K. nana J.L. Yang, Yen *et* Baum	StYP	Y9505	Taxkorgan, Xinjiang, China	EU131911
K. rigidula (Keng *et* S.L. Chen) J.L. Yang, Yen *et* Baum	StYP	ZY3113	Xiahe, Gansu, China	AY740784*
K. thoroldiana (Oliver) J.L. Yang, Yen *et* Baum	StYP	PI531686	Geermu, Qinghai, China	EF396988
K. zhaosuensis J.L. Yang, Yen *et* Baum	StYP	Y2633	Zhaosu, Xinjiang, China	EU131912
Pseudoroegneria (Nevski) Á. Löve				
P. libanotica (Hackel) D.R. Dewey	St	PI228389	Iran	AY730567*
P. spicata (Pursh) Á. Löve	St	PI610986	Utah, United States	AF519158*
P. stipifolia (Czern. *ex.* Nevski) Á. Löve	St	PI325181	Stavropol, Russia	EU131913
P. strigosa ssp. *aegilopoides* (Drobov) Á. Löve	St	PI595164	Xinjiang, China	EF396990
P. tauri (Boiss. & Balansa) Á. Löve	St	PI401323	Iran	EU131914
Agopyron Gaertn.				
A. cristatum (L.) Gaertn.	P	PI19536	Eurasia	AY740791*
A. mongolicum Keng	P	KJ169257	China	AF519117*
Psathyrostachys (Nevski) Á. Löve				
Psa. fragilis (Boiss.) Nevski	Ns	PI243190	Iran	AF519169*
Psa. juncea (Fisch.) Nevski	Ns	PI314521	Russian Federation	AF519170*
Lophopyrum Á. Löve				
Lo. elongatum (Host) Á. Löve	E^e	PI547326	France	AF519166*
Thinopyrum (Savul. & Rayss) Á. Löve				
Th. bessarabicum (Savul. & Rayss) Á. Löve	E^b	PI531712	Estonia, Russia	AF519165*
Australopyrum (Tzvelev) Á. Löve				
Aus. retrofractum (J.W.Vickery) Á. Löve	W	PI531553	Australia	AF519118*
Aus. velutinum (Ness) B.K. Simon	W	D2873-2878	Australia	AF519119*
Hordeum L.				
H. bogdanii Wilensky	H	PI531761	China	AY740789*
H. brevisubulatum (Trin.) Link	H	Y1604	Fuyun, Xinjiang, China	AY740790*
H. jubatum L.	H	H2018	Mexico	AF519123*
Bromus L.				
B. catharticus Vahl	-	S20004	Kunming, Yunnan, China	AY829228*

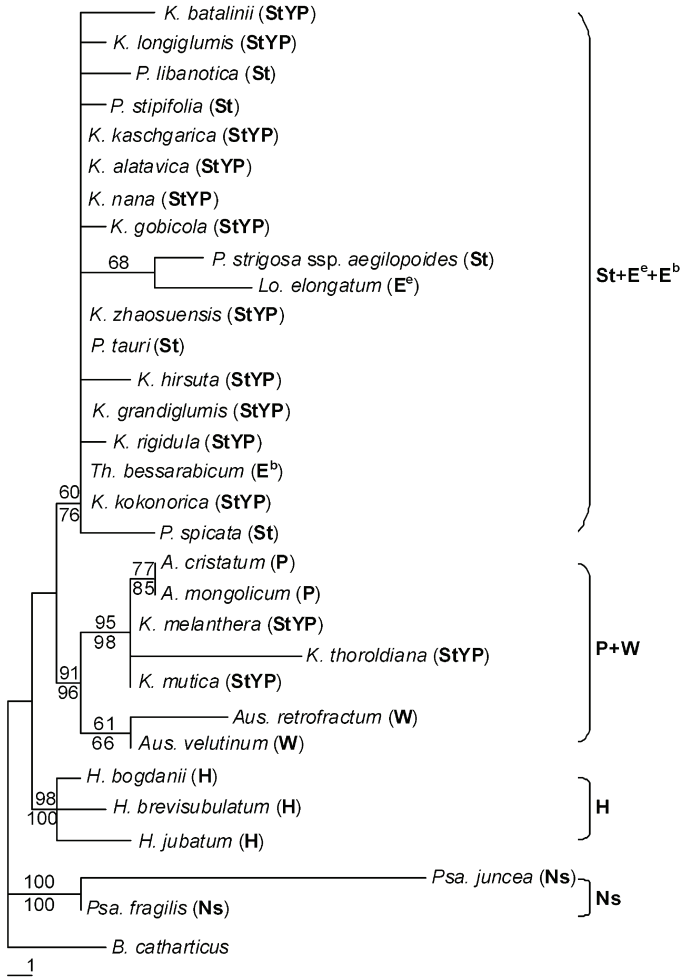

Fig. 1. Strict consensus MP tree of the 106 most parsimonious trees inferred from the *trnL-F* sequence data (tree length = 123, CI = 0.9024, RI = 0.8824). Bootstrap values and Bayesian posterior probability values greater than 50 % are indicated above and below branches respectively. Branch lengths are proportional to the number of nucleotide substitutions, the scale bar at the left corner indicates one substitution. Capital letters in parentheses indicate the genome type of the species.

comprising denaturation at 94 °C for 40 s, annealing at 60 °C for 50 s and extension at 72 °C for 2 min, and a final extension at 72 °C for 7 min. The PCR products were visualized on 1 % agarose gels and then purified by a gel extraction kit *ENZA*^{TM} (*Omega*, Georgia, USA). The purified products were directly sequenced in *Sunbiotech Company* (Beijing, China) and both directions were sequenced. All sequences have been submitted to GenBank (for accession numbers, see Table 1).

The *trnL-F* sequences were edited using *SeqMan* (*DNASTAR* package, Madison, WI, USA) and adjusted manually where necessary. The boundary of *trnL-F* sequences was determined through comparison with the related species from GenBank.

Phylogenetic analysis was conducted using the Maximum Parsimony (MP) method with *PAUP 4.0b10* (Swofford 2003) and Bayesian Inference (BI) with *MrBayes v3.1.2* (Huelsenbeck and Ronquist 2001). In the MP analysis, all transformations were unordered and weighted equally in both data sets and gaps were coded as missing values. 100 replications of random addition were conducted by using heuristic searches with the tree bisection reconnection (TBR) branch swapping and the MULPARS option. The simple sequence addition was used for bootstrap analysis with 1000 replications. In the BI analysis, the model of molecular evolution applied was tested using the Akaike information criterion (AIC) (Akaike 1974). AIC values were calculated and the optimal model was selected using *MrModeltest v2.2* (Nylander 2004) (as implemented by *MrMTgui v1.0*). The model of the general time reversible model (GTR) was chosen. Moreover, an estimated proportion of invariable sites with equal rates had to be assumed according to the AIC (GTR +I). For BI analysis, one cold and three heated Markov chain and Monte Carlo (MCMC) chains were run for 556 000 generations by using the default temperature with trees sampled every 100 generations. The MCMC runs were repeated twice as a safeguard against spurious results. The first 1 000 trees were discarded and the

remaining trees were used to construct a 50 % majority rule consensus tree (BI tree). Examination of the log-likelihoods and the observed consistency among all independent runs suggested that the burn-in periods were long enough for chains to have been become stationary. Nodal support was given by the frequency of the recovered clade, which corresponded to the posterior probability of that clade under the assumed models of sequence evolution (Huelsenbeck and Ronquist 2001).

The lengths of *trnL-F* sequences in this study varied from 850 to 916 bp (average 883 bp). The *trnL-trnF* intergenic spacer comprises 320 - 355 bp (mean 337.5 bp), while the length of *trnL* 3' exon was uniformly 50 bp in all accessions. The average G + C content was 29.3 %.

The alignment matrix of *trnL-F* sequences consisted of 876 characters with 105 variable sites, and of which 34 positions were parsimony informative. The analysis of the *trnL-F* data set produced 106 equally parsimonious trees with a length of 123 steps, consistency index (CI) 0.9024, and retention index (RI) 0.8824. The strict consensus MP tree was shown in Fig. 1. The topologies of the two analyses (MP and BI) were nearly consistent, except the different bootstrap values (BS) of MP and posterior probability (PP) values of BI.

Because of the self-pollinating nature in most species of *Triticeae* (especially in the *Kengyilia* species) and the uniparental mode of inheritance, the chloroplast genes have been widely used to identify the maternal genome donor of a given polyploids in *Triticeae* (Mason-Gamer *et al*. 2002, Liu *et al*. 2006). Yen *et al*. (2006) reported the species of *Kengyilia* originate from natural hybridization of a maternal diploid *Agropyron* with a paternal tetraploid *Roegneria* followed by chromosomal doubling that restored the fertility in the newly formed hexaploid species. Morphologically, some species of *Kengyilia* had dense spikelets which was similar to *Agropyron* species, while others had sparse spikelets which was similar to species in *Roegneria* or *Pseudoroegneria* (Baum *et al*. 1995, Yen *et al*. 2006). The previous molecular evidences showed that *Pseudoroegneria* is apparently the maternal donor in most allopolyploids containing the St genome in *Triticeae* (Jones *et al*. 2000, Redinbaugh *et al*. 2000, Mason-Gamer *et al*. 2002, Liu *et al*. 2006). On the *trnL-F* strict consensus tree, it is evident that there have been multiple maternal donors to *Kengyilia* species. *Agropyron cristatum*, *A. mongolicum* with P genome and *Kengyilia thoroldiana*, *K. melanthera* and *K. mutica* (StYP) formed the subclade P with high support value (BS = 95 %, PP = 98 %). This result suggested that P genome was served as the maternal donor to these species. *Kengyilia batalinii*, *K. nana*, *K. kokonorica*, *K. kaschgarica*, *K. hirsuta*, *K. alatavica*, *K. gobicola*, *K. zhaosuensis*, *K. rigidula*, *K. longiglumis* and *K. grandiglumis* were clustered with the diploid species with St, E^e, E^b genomes in the St+E^e+E^b clade, which suggested that the maternal genome donor to these *Kengyilia* species seems to be St genome.

The St genome is originated from the diploid *Pseudoroegneria* species and the diploid donor species for Y genome has not yet been identified (Dewey 1984, Wang *et al*. 1994). Lu *et al*. (1990) reported the close affinities between the St and Y genomes based on cytological investigations and Liu *et al*. (2006) deduced the same origin of St and Y genomes by analysis of the ITS sequence. In the present study, the St+E^e+E^b clade with weak support value (BS = 60 %, PP = 76 %) contained the species with St, E^e and E^b genomes and most of *Kengyilia* species. Since no obvious Y genome clade was found in this study, it is difficult to rule out either St or Y genome as potential chloroplast donors to the *Kengyilia* species in St+E^e+E^b clade. Therefore, the maternal genome donor to *Kengyilia batalinii*, *K. nana*, *K. kokonorica*, *K. kaschgarica*, *K. hirsuta*, *K. alatavica*, *K. gobicola*, *K. zhaosuensis*, *K. rigidula*, *K. longiglumis* and *K. grandiglumis* is not P but St or Y genome.

Molecular studies have successfully revealed the evolutionary history of polyploids and phylogenetic relationships in plants (Mason-Gamer *et al*. 2002, Liu *et al*. 2006, Das *et al*. 2007, Yang *et al*. 2008, Zeng *et al*. 2008). The cpDNA *trnL-F* sequence region has recently proved to be of great significance at high taxonomic levels for inferring (Wurdack *et al*. 2005). In the present study, the *trnL-F* sequence region can not provide sufficient phylogenetic informative sites to reveal relationships among *Kengyilia* species. However, this region inferred the relationships among the seven basic genomes in perennial *Triticeae*. In support of the previous study (Hsiao *et al*. 1995, Mason-Gamer *et al*. 2002, Liu *et al*. 2006), diploid species of P and W genome clustered together with high support value (BS = 91 %, PP = 96 %), which suggested the close affinities between P and W. The species with genomes St, E^e and E^b were clustered together with an insertion of a short sequence 'AATGAT' from positions 261-266 in the alignment, which indicated the close relationships between St, E^e and E^b genomes. P, W, St, E^e and E^b were distinct from the genome Ns and H. The diploid H genome species shared insertion of a short sequence AGGAA from position 650 - 654 in the alignment, which was an important feature of H genome.

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