Maternal donors of polyploids in *Pseudoroegneria* (Poaceae: Triticeae) and related genera inferred from chloroplast *trnL*-F sequences

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Received: 16.10.2008

Abstract: To investigate the maternal donors and phylogenetic relationships of polyploids in *Pseudoroegneria* and related genera, the chloroplast *trnL*-F sequences of 31 Triticeae accessions were analyzed. The substitution saturation analysis of *trnL*-F sequences in this study suggested that they are suitable for phylogenetic analysis. In the *trnL*-F sequences tree, 4 major clades were formed: (a) the St/E clade comprised all of the *Pseudoroegneria* species together with species in *Roegneria*, *Elytrigia*, *Douglasdeweya* and *Lophopyrum*; (b) *A. cristatum*, *A. mongolicum*, and *A. pectinatum* subsp. *retrofractum* formed the P/W clade; (c) the Ns clade included species in *Psathyrostachys*; and (d) the H clade consisted of *Hordeum* species. The results suggested that: (a) diploid *Pseudoroegneria* species were the maternal donors of St-containing polyploid species in *Pseudoroegneria*, *Roegneria*, *Douglasdeweya*, and *Elytrigia*, and the *trnL*-F sequences were highly similar among them; (b) the *trnL*-F sequences of *Agropyron* species and *Australopyrum* species were similar, and the P genome was closely related to the W genome; and (c) the *trnL*-F sequences of species with the H or Ns genomes diverged greatly from that of species with the St, E, P, or W genomes.

Key words: *Pseudoroegneria*, *Douglasdeweya*, *Roegneria*, *Elytrigia*, chloroplast *trnL*-F

**Pseudoroegneria** (Poaceae: Triticeae) ve ilişkili cinslerde kloroplast *trnL*-F dizilerinden elde edilen poliploitlerin maternal vericileri

Özet: *Pseudoroegneria* ve ilişkili cinslerde, poliploitlerin filogenetik ilişkilerini ve maternal vericilerini araştırmak için, 31 Triticeae örneğinin kloroplast *trnL*-F dizileri analiz edildi. Bu çalışmada *trnL*-F dizilerinin yer değişim doygunluk analizleri filogenetik analizler için uygunduğunu önerdi. *trnL*-F dizilerine ait ağaçta, dört büyük dal şekillendi. (a) St/E dalı *Roegneria*, *Elytrigia*, *Douglasdeweya* ve *Lophopyrum* ile birlikte tüm *Pseudoroegneria* türlerini içirdi; (b) *A. cristatum*, *A. mongolicum* ve *A. pectinatum* subsp. *retrofractum* türleri P/W dalını şekillendirdi; (c) Ns dalı *Psathyrostachys* türlerini içirdi; (d) H dalı *Hordeum* türlerinden oluştu. Sonuçlar (a) *trnL*-F dizileri yüksek oranda benzerlik gösteren *Pseudoroegneria*, *Roegneria*, *Douglasdeweya* ve *Elytrigia* cinslerini içinde yer alan St içeren poliploid türlerin maternal vericilerinin diploid *Pseudoroegneria* türleri olduğu; (b) P ve W genomlarının çok yakın ilişkili, *Agropyron* ve *Australopyrum* türlerinin *trnL*-F dizilerinin benzer olduğunu; (c) H veya Ns genomlu türlerin *trnL*-F dizilerinin büyük ölçüde St, E, P veya W genomlu türlerden farklı çıktığını önerdi.

Anahtar sözcükler: *Pseudoroegneria*, *Douglasdeweya*, *Roegneria*, *Elytrigia*, kloroplast *trnL*-F
Introduction

*Pseudoroegneria* is a genus in Triticeae (Poaceae) with *Pseudoroegneria strigosa* (M. Bieb.) Á. Löve as the type species (1). It consists of about 15 species that are built around 1 genome designated St, which is the donor genome of the species in *Douglasdeweya* (StP), *Roegneria* (StY), *Elytrigia* (ESt), *Elymus* (StH), *Kengyilia* (StYP), and *Pascopyrum* (StHNsXm) (2-6). The genus is distributed in the northern hemisphere, with its species occurring on open rocky hillsides from the Middle East and Transcaucasia across Central Asia and northern China to western North America. *Pseudoroegneria* grasses are exceptionally drought tolerant and have excellent forage quality (3).

Morphologically, *Pseudoroegneria* species are caespitose, long-anthered, cross-pollinating perennials (1). However, similar morphological characters are also found in several related genera, such as *Elytrigia* and *Douglasdeweya* (3,5). Cytologically, *Pseudoroegneria* species are diploids or tetraploids, and possess St, StSt, StP, or ESt genomes. Based on the ITS data analysis, *Pseudoroegneria* species are closely related to those in 3 Triticeae genera, namely *Peridictyon*, *Heteranthelium*, and *Dasyphyrum* (7). Löve and Dewey suggested that the species are closely related to those in 3 Triticeae species (23,25). The chloroplast trnL-F sequences were sequenced and analyzed. The chloroplast DNA (cpDNA) sequences, particularly the noncoding regions such as the intron of *trnL* (UAA) and the intergenic spacer of *trnL* (UAA)-*trnF* (GAA), are valuable sources of markers for identifying the maternal donors of polyploids, with additional capacity to reveal phylogenetic relationships of related species (23,25). The chloroplast trnL-F sequence has successfully demonstrated the maternal origin of polyploids in *Elymus* (25,26), while it is not estimated among polyploids in *Pseudoroegneria* and related genera. To understand the maternal donors and phylogenetic relationships of polyploids in *Pseudoroegneria* and related genera, the chloroplast trnL-F sequences were sequenced and analyzed. The aims were: (a) to investigate the maternal donors of polyploids in *Pseudoroegneria* and related genera; and (b) to evaluate the phylogenetic relationships of species among *Pseudoroegneria*, *Douglasdeweya*, *Roegneria*, *Elytrigia*, and *Lophopyrum*.

Materials and methods

Plant materials

A total of 31 Triticeae accessions, including 13 *Pseudoroegneria* (St), 4 *Roegneria* (StY), 3 *Elytrigia* (E'St), 2 *Douglasdeweya* (PSt), 2 *Lophopyrum* (E' and E''), 2 *Agropyron* (P), 1 *Australopyrum* (W), 2
Psathyrostachys (Ns), and 2 Hordeum (H) accessions, were used in this study. Bromus catharticus Vahl was used as an outgroup. All of the seed materials were kindly provided by the American National Plant Germplasm System (Pullman, Washington, USA) and the Triticeae Research Institute, Sichuan Agricultural University, China. These seeds were germinated and grown in the perennial nursery. The mature plants were carefully identified by Professors Chi Yen, Junliang Yang, and Yonghong Zhou. The taxa, accession numbers, genomic constitutions, geographic origins, and GenBank accession numbers are listed in the Table. The nomenclature and genome symbols of the species used in this study follow the opinions of Löve (2), Dewey (3), Wang et al. (12), and Yen et al. (5).

DNA extraction and purification

The leaf samples for each material were collected from mature plants in the perennial nursery of the Triticeae Research Institute and ground in liquid nitrogen in a 1.5 mL microfuge tube. DNA was extracted and purified with a slight modification of the cetyltrimethylammonium bromide (CTAB) procedure outlined by Doyle and Doyle (27).

trnL-F amplification and sequencing.

The amplification of trnL-F regions was done using primers r (5’-CGAAATCGGTAGACGCTACG-3’) and f (5’-ATTTGAACTGGTGACACGAG-3’) (28). The PCR reaction was carried out in a total volume of 25 μL, containing 1× reaction buffer, 1.5 mM MgCl₂, 0.5 μM of each primer, 200 μM of each dNTP (TakaRa Biotechnology (Dalian) Co., Ltd., Dalian, China), 0.5 units of ExTaq Polymerase (TakaRa), and sterile water to the final volume. The thermocycling profile consisted of an initial denaturation at 94 °C for 3 min, followed by 30 cycles of 40 s at 94 °C, 50 s at 60 °C, 2 min at 72 °C, and a final extension of 8 min at 72 °C. PCR reactions of each accession were carried out in an ABI 9700 thermal cycler (Applied Biosystems, CA, USA). Amplification products were purified using the Gel Extraction Kit (50) (OMEGA, GA, USA). The purified products were directly sequenced in both directions by Sunbiotech Company (Beijing, China). The sequences used in this study have been submitted to NCBI (http://www.ncbi.nlm.nih.gov).

Sequence alignment and phylogenetic analysis

The boundaries of the trnL-F regions were determined by comparison with the trnL-F sequence of Pseudoroegneria libanotica (Hackel) D.R. Dewey (GenBank accession number AY730567) (25). The trnL-F sequence alignment was executed with the Clustal X program and adjusted manually where necessary (29). Gaps were coded as binary characters by their presence/absence and were used for the phylogenetic analyses. DAMBE 4.5.67 (30) was used to test substitution saturation.

PAUP* 4.0b10 (31) was used to find the most parsimonious trees by performing a heuristic search with tree-bisection-reconnection (TBR) branch swapping, the MULTREES option, ACCTRAN optimization, and 100 random addition replicates. Topological robustness was assessed by bootstrap analysis with 1000 replications using simple taxon addition.

Results

Sequence analysis of trnL-F regions

The trnL-F fragment sequenced in this study included 4 regions: (1) the partial trnL intron, (2) the trnL 3’ exon, (3) the trnL-trnF intergenic spacer, and (4) the partial trnF exon with 40 base pairs (bp). The length of the sequenced chloroplast trnL-F varied from 859 to 882 bp in all accessions. The average of the G + C content was 29%. Using the parsimony criterion, 47 of the 108 variable sites were parsimoniously informative, including the polymorphisms introduced by insertions/deletions. The analysis of substitution saturation for the trnL-F sequence was conducted and is shown in Figure 1.

Phylogenetic analysis of the chloroplast trnL-F sequences

The chloroplast trnL-F sequences of polyploids related to Pseudoroegneria and their putative diploid donor species were included for phylogenetic analysis. Maximum parsimony (MP) analysis resulted in 154 equally most parsimonious trees with 134 steps, a consistency index of 0.8538, and a retention index of 0.7982. The strict consensus tree constructed by MP is shown in Figure 2. The percentage of bootstrap values is indicated above the branches.
Maternal donors of polyploids in *Pseudoroegneria* (Poaceae: Triticeae) and related genera inferred from chloroplast trnL-F sequences

### Table. Materials used in the trnL-F analysis.

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Accession No.</th>
<th>Genome</th>
<th>Geographic Origin</th>
<th>GenBank Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>P. cognata</em> (Hack.) Á. Löve</td>
<td>PI 531720</td>
<td>St</td>
<td>Estonia, Russian Federation</td>
<td>EU139482</td>
</tr>
<tr>
<td>2</td>
<td><em>P. geniculata</em> (Trin.) Á. Löve</td>
<td>PI 565009</td>
<td>StSt</td>
<td>Russian Federation</td>
<td>EU139485</td>
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<tr>
<td>3</td>
<td><em>P. geniculata</em> subsp. pruinifera* (Nevski) Á. Löve</td>
<td>PI 547374</td>
<td>—</td>
<td>Ural, Russian Federation</td>
<td>EU139483</td>
</tr>
<tr>
<td>4</td>
<td><em>P. geniculata</em> subsp. scythica* (Nevski) Á. Löve</td>
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<td>E'St</td>
<td>Russian Federation</td>
<td>EU139484</td>
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<tr>
<td>5</td>
<td><em>P. gracillima</em> (Nevski) Á. Löve</td>
<td>PI 440000</td>
<td>St</td>
<td>Stavropol, Russian Federation</td>
<td>EU139486</td>
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<td>6</td>
<td><em>P. kosaninii</em> (Nabelek) Á. Löve</td>
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<td>St</td>
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</tr>
<tr>
<td>8</td>
<td><em>P. spicata</em> (Pursh) Á. Löve</td>
<td>PI 610986</td>
<td>St</td>
<td>Utah, United States</td>
<td>AF519158*</td>
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<tr>
<td>9</td>
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<td>PI 232124</td>
<td>StSt</td>
<td>Washington, United States</td>
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<tr>
<td>10</td>
<td><em>P. stipifolia</em> (Czern. ex Nevski) Á. Löve</td>
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<td>St</td>
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<td>EF396989</td>
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<tr>
<td>11</td>
<td><em>P. strigosa</em> subsp. aegilopoides* (Drobov) Á. Löve</td>
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<td>Xinjiang, China</td>
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<td>12</td>
<td><em>P. tauri</em> (Boiss. &amp; Balansa) Á. Löve</td>
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<td>Iran</td>
<td>EF396991</td>
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<td>St</td>
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<td>14</td>
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<td>StSt</td>
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<td>AY730568*</td>
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<td>PI 531717</td>
<td>E'St</td>
<td>Estonia, Russian Federation</td>
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<tr>
<td>19</td>
<td><em>L. elongatum</em> (Host) Á. Löve</td>
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<td><em>Douglasdeveya</em> C. Yen, J.L. Yang &amp; B.R. Baum</td>
<td>Z2005</td>
<td>StSt</td>
<td>Changdu, Tibet, China</td>
<td>AY730568*</td>
</tr>
<tr>
<td>21</td>
<td><em>D. deweyi</em> (K.B. Jensen, S.L. Hatch &amp; J.K. Wipff)</td>
<td>PI 531756</td>
<td>PSt</td>
<td>Caucasus, Russian Federation</td>
<td>EU139478</td>
</tr>
<tr>
<td>22</td>
<td><em>D. wangii</em> C. Yen, J.L. Yang &amp; B. R. Baum</td>
<td>PI 380645</td>
<td>PSt</td>
<td>Iran</td>
<td>EU139479</td>
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<tr>
<td>23</td>
<td><em>L. pectinatum</em> subsp. retrofractum* (J.W. Vickery) Á. Löve</td>
<td>H10154</td>
<td>P</td>
<td>Altai, Xinjiang, China</td>
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<td>24</td>
<td><em>A. cristatum</em> (L.) Gaertner</td>
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<td>25</td>
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<td>PI 531717</td>
<td>E'St</td>
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<td>26</td>
<td><em>Australopyrum</em> (Tzvelev) Á. Löve</td>
<td>PI 531717</td>
<td>E'St</td>
<td>Estonia, Russian Federation</td>
<td>AF519165*</td>
</tr>
</tbody>
</table>

*GenBank accession number was published previously by GenBank (http://www.ncbi.nlm.nih.gov).*
Four major clades (St/E, P/W, H, and Ns) were formed. The H clade consisted of Hordeum species. The Ns clade included species of Psathyrostachys, Agropyron cristatum (L.) Gaertner, A. mongolicum, and Aegilops aestivum subsp. retrofractum (J.W. Vickery) Á. Löve formed the P/W clade. The St/E clade, the largest, comprised all of the Pseudoroegneria species (Pseudoroegneria cognata (Hack.) Á. Löve, Pseudoroegneria gracillima (Nevski) Á. Löve, Pseudoroegneria stipifolia (Czern. ex Nevski) Á. Löve, Pseudoroegneria strigosa subsp. aegilopoides (Drobow) Á. Löve, Pseudoroegneria tauri (Boiss. & Balansa) Á. Löve, P. libanotica, Pseudoroegneria stipifolia (Czern. ex Nevski) Á. Löve, Pseudoroegneria stipifolia subsp. scythica, E. caespitosa, E. caespitosa subsp. nodosa, E. intermedia, R. alashanica, R. eleytrigoides, R. magnicaespes, R. grandis, D. wangii, and D. deweyi contained the St genome (3,8-10,17,36). They were clustered together with diploid Pseudoroegneria species (P. spicata, P. libanotica, P. tauri, P. gracillima, P. stipifolia, P. cognata, and P. strigosa subsp. aegilopoides). This indicated that the trnL-F sequences were highly similar among species in Pseudoroegneria, Roegneria, Douglasdeweyea, and Elytrigia, and diploid Pseudoroegneria species were suggested as their chloroplast genome donors. The genomic constitutions of P. geniculata subsp. pruinifera and P. kosaninii are unknown, and they were also in one clade together with diploid Pseudoroegneria species. This indicated that they have the St genome, originated from diploid Pseudoroegneria species. Based on the ndhF data, Redinbaugh et al. (37) suggested that there is a strong preference for cpDNA inheritance from the St-containing parent in hybridizations between Triticeae species, and the St-containing parent as the female may be more successful. Similar results were also obtained in this study, and all of the polyploid species were clustered together with diploid Pseudoroegneria species.

A. mongolicum is indicated as the maternal donor of D. wangii based on morphological comparison (8). In the present analysis, D. wangii and A. mongolicum were in different clades, and D. wangii was closely related to Pseudoroegneria species. This provided strong evidence that the maternal donor of D. wangii was derived from diploid Pseudoroegneria species, and that the Agropyron species was the paternal donor of D. wangii. Jensen et al. (9) suggested that P. stipifolia is most likely the maternal donor of D. deweyi. In this study, the St genome served as the
Maternal genome donor of *D. deweyi*. However, it is not known whether *P. stipifolia* is the maternal donor of *D. deweyi*.

Molecular studies have successfully revealed the evolutionary history of polyploids and phylogenetic relationships in plants (23-25). The cpDNA sequence
region has recently proven to be of great significance at high taxonomic levels for inferring the maternal parents of polyploid species (38). The substitution saturation analysis of trnL-F sequences in this study suggested that it is suitable for phylogenetic analysis. *L. elongatum* and *L. bessarabicum* have E<sup>e</sup> and E<sup>b</sup> genomes, respectively. They were clustered together with diploid *Pseudoroegneria* species. This indicated that their trnL-F sequences were highly similar, and the E genome (E<sup>e</sup> and E<sup>b</sup>) was closely related to the St genome. *Agropyron* species and *Australopyrum* species were in one clade. This indicated that their trnL-F sequences were similar, and the P genome was closely related to the W genome. These results were consistent with previous molecular and cytological studies (23-25,39). *Hordeum* species and *Psathyrostachys* species were clustered into different clades, respectively, which revealed that the trnL-F sequences of species with H or Ns genomes diverged greatly from those of species with St, E, P or W genomes. Mason-Gamer et al. (23) reported similar results based on molecular evidence from rpoA, tRNA spacers, restriction sites, and their combined data.

**Acknowledgements**

The authors are thankful to the Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT), China (No. IRT 0453); the National Natural Science Foundation of China (No. 30670150, 30470135); the Science and Technology Bureau of Sichuan Province, China; and the Education Bureau of Sichuan Province, China for the financial support. We particularly thank the American National Plant Germplasm System for providing seeds.

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**References**

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