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Genome analysis of seven species of *Kengyilia* (Triticeae: Poaceae) with FISH and GISH

Quanwen Dou, Richard R.-C. Wang, Yuting Lei, Feng Yu, Yuan Li, Haiqing Wang, and Zhiguo Chen

Abstract: The genome compositions and genetic relationships of seven species of *Kengyilia* were assessed using a sequential fluorescence in situ hybridization (FISH) and genomic in situ hybridization (GISH) technique. Five species, *K. kokonorica*, *K. rigidula*, *K. hirsuta*, *K. grandiglumis*, and *K. thoroldiana*, are native to Qinghai (China). The other two, *K. alatavica* and *K. batalinii*, are distributed in Xinjiang (China) and Kyrgyzstan, respectively. Each chromosome could be easily identified using chromosome markers (45S rDNA, 5S rDNA, pAs1, and AAG repeats) by FISH and allocated to the **St**, **P**, or **Y** genome by GISH. Molecular karyotype comparison indicated that *K. alatavica* and *K. batalinii* were distinct from the Qinghai species in all three genomes. These results support that the species of *Kengyilia* from Central Asia and the Qinghai–Tibetan plateau have independent origins. Genomic differentiation was still detected among the species of *Kengyilia* from Qinghai. Specifically, a common species-specific pericentric inversion was identified in both *K. grandiglumis* and *K. thoroldiana*, and an identical **St-P** non-Robertsonian translocation was frequently detected in *K. hirsuta*. The Qinghai species formed three genetic groups, *K. kokonorica*–*K. rigidula*, *K. hirsuta*, and *K. grandiglumis*–*K. thoroldiana*. The possible role of species-specific inversions and translocations in the evolution of **StPY** species is discussed.

Key words: species of *Kengyilia*, molecular karyotype, karyotype evolution.

Résumé : La composition du génome et les relations génétiques au sein de sept espèces du genre *Kengyilia* ont été étudiées au moyen d'analyses FISH (hybridation in situ en fluorescence) séquentielles et GISH (hybridation génomique in situ). Cinq de ces espèces, *K. kokonorica*, *K. rigidula*, *K. hirsuta*, *K. grandiglumis* et *K. thoroldiana* sont originaires du Qinghai (Chine). Les deux autres, *K. alatavica* et K. *batalinii*, sont retrouvées respectivement dans le Xinjiang (Chine) et le Kyrgyzstan. Chacun des chromosomes a été aisément distingué au moyen de marqueurs chromosomiques (ADNr 45S, ADNr 5S, pAS1 et répétitions AAG) par analyse FISH et l'appartenance au génome **St**, **P** ou **Y** a été établie par analyse GISH. La comparaison des caryotypes moléculaires a révélé que le *K. alatavica* et le *K. batalinii* sont des espèces distinctes des espèces du Qinghai pour tous les génomes. Ces résultats appuient l'hypothèse voulant que les espèces provenant d'Asie centrale et du Qinghai–Plateau tibétain auraient des origines indépendantes. De la différentiation génomique a aussi été détectée au sein des espèces de *Kengyilia* du Qinghai. Spécifiquement, une inversion péricentrique spécifique commune a été identifiée chez le *K. grandiglumis* et le *K. thoroldiana*, tandis qu'une translocation non-robertsonienne **St-P** identique a été détectée fréquemment chez le *K. hirsuta*. Les espèces du Qinghai ont formé trois groupes, *K. kokonorica*–*K. rigidula*, *K. hirsuta* et *K. grandiglumis*–*K. thoroldiana*. Les auteurs discutent des rôles possibles qu'auraient joués ces inversions et translocations spécifiques des espèces dans l'évolution des espèces à génomes **StPY**. [Traduit par la Rédaction]

Mots-clés : espèces de *Kengyilia*, caryotype moléculaire, évolution caryotypique.

Introduction

Species of *Kengyilia* C. Yen & J.L. Yang, a perennial genus in the tribe Triticeae of the family Poaceae, are commonly distributed in central Asia and the Qinghai–Tibetan plateau and grow in meadows, steppes, the fringes of forests, and also semideserts or extremely dry deserts at altitudes from 1100 to 4750 m (Yang et al. 1992). To date, about 32 species and subspecies have been identified in this genus (Cai and Zhi 1999). The origin of *Kengyilia* was first suggested to be from natural amphiploids between tetraploid *Roegneria* K. Koch (**StY** genome) and diploid *Agropyron cristatum* (L.) Gaertn. (**P** genome). Species of *Roegneria* and *Agropyron* are sympatric in many areas (Yang et al. 1992). Cytological evidence confirmed the polyploid nature of *Kengyilia* with three different genomes, **St**, **Y**, and **P** (Jensen, 1990, 1996). Thus, the genome constitution of *Kengyilia* was designated as **StPY**, of which the **St** and **P** genomes are derived from *Pseudoroegneria* (Nevski) Á. Love and *Agropyron*, respectively, although the origin of the **Y** genome is still unknown (Wang et al. 1994).

The evolutionary differentiation of species of *Kengyilia* was suggested to be associated with its geographical origin in Central Asia and the Qinghai–Tibetan plateau, based on evidence from random amplified polymorphic DNA (RAPD) (Zhou et al. 2000), random amplified microsatellite polymorphism (RAMP) (Zhang et al. 2005), C-banded karyotypes (Zeng et al. 2008*a*), and ITS sequence data (Zeng et al. 2008*b*). Investigation of the evolutionary dynamics of the *Pgk*1 gene in *Kengyilia* and its diploid relatives further

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Table 1. Plant materials used in this study.

Species	Genome symbol	Identification No.	Source	Altitude (m)
Agropyron mongolicum	**P**	—	Inner Mongolia	1000
Agropyron cristatum	**P**	—	Haiyan, Qinghai	3295
Pseudoroegneria stipifolia	**St**	PI 313960	FRRL	—
Kengyilia kokonorica	**StPY**	YL 006	Gonghe, Qinahai	3100
		NW 011	Gangcha, Qinghai	3309
Kengyilia rigidula	**StPY**	YL 002	Guinan, Qinghai	3200
		NW 005	Gangcha, Qinghai	3232
Kengyilia hirsuta	**StPY**	NW 055	Xinghai, Qinghai	3115
Kengyilia grandiglumis	**StPY**	NW 017	Haiyan, Qinghai	3295
		HS 001	Guinan, Qinghai	3400
Kengyilia thoroldiana	**StPY**	SL 001	Maduo, Qinghai	4200
		SL 002	Tongde, Qinghai	3285
Kengyilia alatavica	**StPY**	PI 499588	FRRL (Xingjiang)	—
Kengyilia batalinii	**StPY**	PI 531562	FRRL (Kyrgyzstan)	—

Note: FRRL, Forage and Range Research Laboratory, Utah State University, Logan, Utah.

supported that the species of *Kengyilia* from Central Asia and the Qinghai–Tibetan plateau have independent origins, and that geographically differentiated **P**-genome donors could be attributed to geographical differentiation of species of *Kengyilia* via independent origins (Fan et al. 2012).

Molecular cytogenetic analysis using fluorescence in situ hybridization (FISH) and genomic in situ hybridization (GISH) has provided powerful methods to detect karyotype variation. Comparisons of the FISH-based karyotypes of closely related species have provided cytogenetic evidence for evolutionary relationships (Schwarzacher 2003; Jiang and Gill 2006). GISH, using the total genomic DNA of an analyzer (a species with a known genome) as a probe, can reveal intergenomic translocations, as well as the presence, or absence, of the analyzer genome in polyploid species (Ørgaard and Heslop-Harrison 1994). The sequential FISH and GISH technique, combining the information of FISH and GISH together, has proven to be powerful for detecting genome compositions and variation in Triticeae polyploid species (Dou et al. 2009, 2011). In the genus *Kengyilia*, molecular karyotyping has only been carried out on the species *K. grandiglumis* with the satellite sequence pAs1 and the microsatellite GAA sequence by the GISH–FISH method (Wang et al. 2010).

In this study, a molecular cytological investigation was conducted on seven species of *Kengyilia*: *K. kokonorica* (Keng) J.L. Yang, C. Yen & B.R. Baum; *K. rigidula* (Keng) J.L. Yang, C. Yen & B.R. Baum; *K. hirsuta* (Keng) J.L. Yang, C. Yen & B.R. Baum; *K. grandiglumis* (Keng) J.L. Yang, C. Yen & B.R. Baum; *K. thoroldiana* (Oliv.) J.L. Yang, C. Yen & B.R. Baum; *K. alatavica* (Drobow) J.L. Yang, C. Yen & B.R. Baum; and *K. batalinii* (Krasn.) J.L. Yang, C. Yen & B.R. Baum. First, individual chromosomes were characterized with FISH probes of 5S rDNA, 45S rDNA, pAs1, and AAG repeats. Secondly, the chromosomes were allocated to the different genomes, **St**, **Y**, and **P**, by GISH. This sequential FISH and GISH method allowed us to present detailed molecular karyotypes for these seven species of *Kengyilia* plus two species of *Agropyron*. Our results could be very helpful in elucidating the origins and evolution of the **St**, **P**, and **Y** genomes, and relationships among these species at the chromosomal level. Additionally, *K. grandiglumis* and *K. thoroldiana* have been selected and used as potent grasses for ecological recovery in the Qinghai–Tibet plateau. This genomic information could be very useful for germplasm enhancement of these species.

Materials and methods

Plant materials

Five species of *Kengyilia* and *A. cristatum* were collected in Qinghai, China. *Agropyron mongolicum* was collected in Inner Mongolia, China, and *Pseudoroegneria stipifolia* and two other species of *Kengyilia* were kindly provided by the Forage and Range Research Laboratory (FRRL), Utah State University, Logan, Utah (Table 1).

Probe preparation

Genomic DNAs of *P. stipifolia* and *A. cristatum* were fragmented by autoclaving following the procedures of Dou et al. (2009). The 5S rDNA was amplified by polymerase chain reaction (PCR) using genomic DNA of *P. stipifolia* as described by Fukui et al. (1994). The treated genomic DNAs and amplified 5S rDNA were labeled with tetramethyl-rhodamine-5-dUTP (red) or fluorescein-12-dUTP (green) (Roche Diagnostics, Germany) by a random primer labeling method (Prime-it Fluor Fluorescence Labeling Kit, Agilent Technologies). pAs1 (including a repetitive sequence from *Aegilops squarrosa*; Rayburn and Gill 1986) and pWrrn (including fragments of wheat 45S rDNA) were provided by H. Tsujimoto (Tottori University, Japan) and were labeled with tetramethyl-rhodamine–5-dUTP (red) by a nick-translation method. A 30-base length microsatellite DNA, $(AAG)_{10}$, was synthesized with a fluorescein-12-dUTP in the 3′-end.

Chromosome preparation

Root tips with a length of 1–2 cm were collected, pretreated in ice-cold water at 0–4 °C for 20–24 h, fixed in ethanol – glacial acetic acid (3:1, *v*/*v*) for 24 h at room temperature, and then stored at 4 °C in the refrigerator. Each root tip was squashed in a drop of 45% acetic acid. Slides were observed with a phase contrast microscope (Olympus). The slides with well-spread chromosomes were kept at −80 °C in the freezer for more than half an hour, and the cover glass was removed with a blade. The slide preparations were air dried for further processing.

In situ hybridization and fluorescence microscopy

Sequential FISH and GISH experiments were carried out as described by Dou et al. (2009, 2011) with minor modifications. Each hybridization was carried out at 37 °C for over 24 h in 10 μL per slide of a mixture containing 10–15 ng of each labeled probe DNA, 5–10 mg of sheared salmon sperm DNA, 50% formamide, 2× SSC, and 10% dextran sulfate. Sequential hybridization was conducted without denaturation after post-hybridization washing with distilled water. GISH probing with labeled genomic DNAs of *A. cristatum* (**P** genome) and *P. stipifolia* (**St** genome) was carried out simultaneously. No blocking DNAs were added in the GISH probing steps. Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Images were captured with a cooled CCD camera (Photometrics CoolSNAP) under a fluorescence microscope (Leica) and processed with the Meta Imaging System (Universal Imaging Corporation). Finally, images were adjusted with Adobe Photoshop 6.0 for contrast and background optimization.

Results

Sequential FISH and GISH experiments were conducted on two species of *Agropyron* and seven species of *Kengyilia*. First, the 45S rDNA and 5S rDNA hybridization sites were physically mapped

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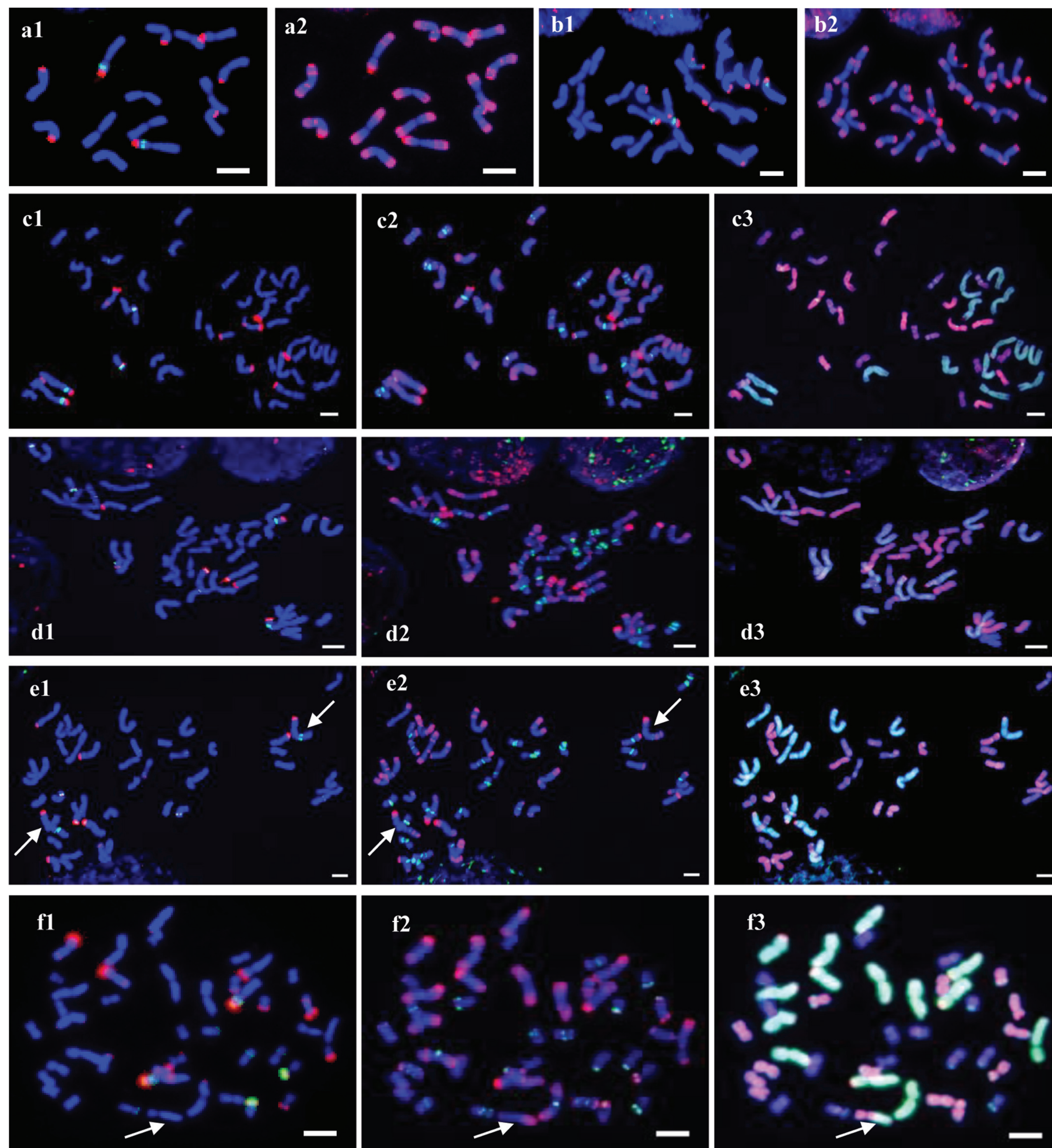
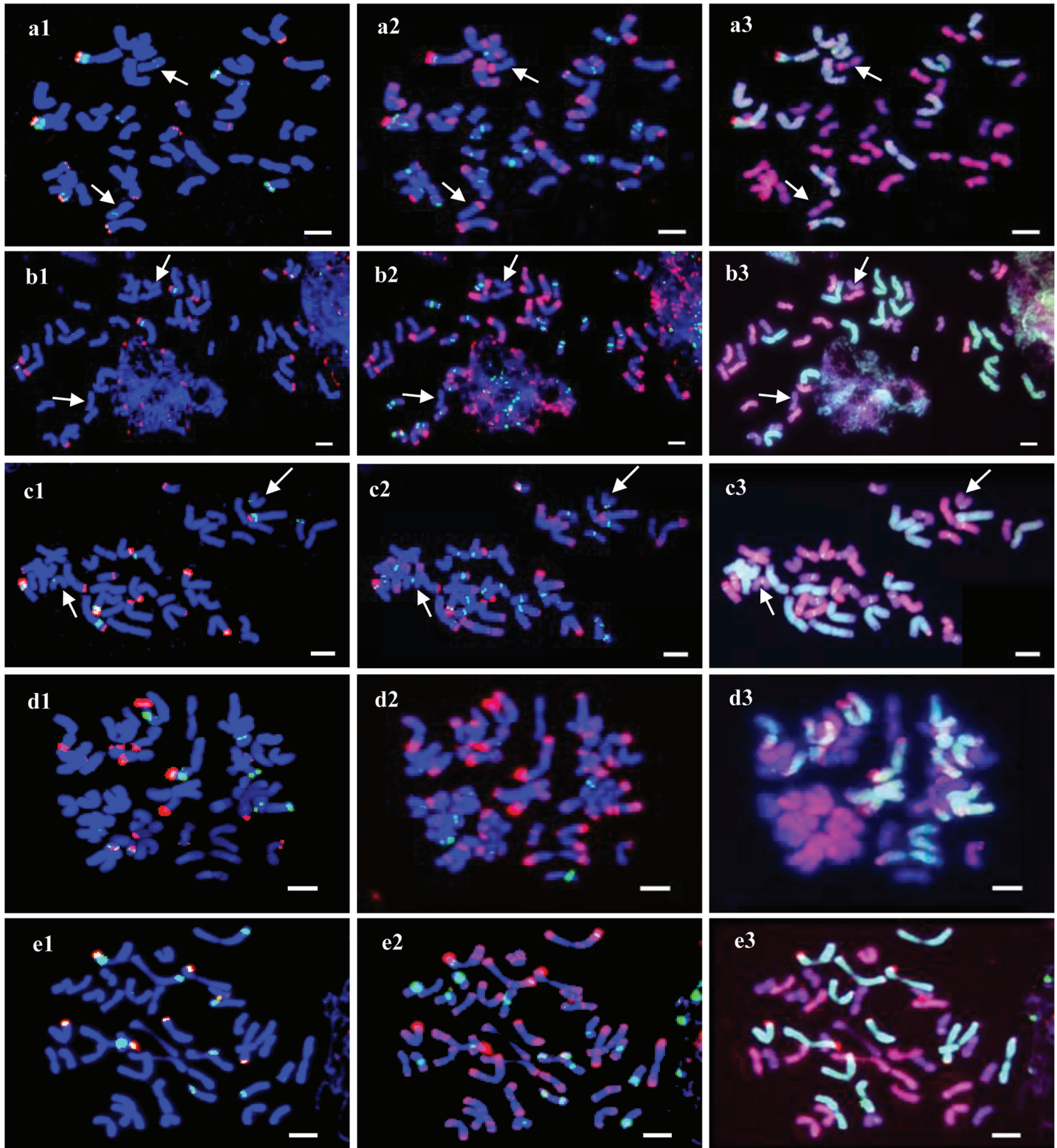
Fig. 1. Sequential fluorescence in situ hybridization (FISH) and genomic in situ hybridization (GISH) patterns of mitotic metaphase chromosomes in (*a*) *Agropyron mongolicum*; (*b*) *A. cristatum*; (*c*) *Kengyilia kokonorica*; (*d*) *K. rigidula*; (*e*) *K. rigidula*; (*f*) *K. hirsuta*. Figure parts *a1*, *b1*, *c1*, *d1*, *e1*, *f1*: First FISH probed with 45S DNA (red) and 5S rDNA (green). Figure parts *a2*, *b2*, *c2*, *d2*, *e2*, *f2*: Sequential FISH probed with pAs1 (red) and $(AAG)_{10}$ (green). Figure parts *c3*, *d3*, *e3*, *f3*: Sequential GISH probed with genomic DNAs of *A. cristatum* (green) and *Pseudoroegneria stipifolia* (red). Chromosomes of the **P**, **St**, and **Y** genomes were stained with green, red, and pink, respectively. The arrows indicate a pericentric inversion in *e1* and *e2*, and a translocation in *f1*, *f2*, and *f3*.



(Figs. 1*a1*–1*f1* and 2*a1*–2*e1*). Second, FISH was carried out using AAG and pAs1 repeats on the same cells (Figs. 1*a2*–1*f2* and 2*a2*–2*e2*). Subsequently, GISH was applied to the cells of the seven species of *Kengyilia* using genomic DNA probes of *A. cristatum* (**P** genome) and *P. stipifolia* (**St** genome). Thus, each chromosome of the two species of *Agropyron* were clearly characterized, and all chromosomes of the species of *Kengyilia* were clearly classified into the genomes **St**, **P**, or **Y**. Each chromosome of a different genome could only be

Fig. 2. Sequential fluorescence in situ hybridization (FISH) and genomic in situ hybridization (GISH) patterns of mitotic metaphase chromosomes in (*a*) *Kengyilia grandiglumis*; (*b*) *K. grandiglumis*; (*c*) *K. thoroldiana*; (*d*) *K. alatavica*; (*e*) *K. batalinii*. Figure parts *a1*, *b1*, *c1*, *d1*, *e1*: First FISH probed with 45S DNA (red) and 5S rDNA (green). Figure parts *a2*, *b2*, *c2*, *d2*, *e2*: Sequential FISH probed with pAs1 (red) and $(AAG)_{10}$ (green). Figure parts *a3*, *b3*, *c3*, *d3*, *e3*: Sequential GISH probed with genomic DNAs of *Agropyron cristatum* (green) and *Pseudoroegneria stipifolia* (red). Chromosomes of the **P**, **St**, and **Y** genomes were stained with green, red, and pink, respectively. The arrows indicate a pericentric chromosome inversion.



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discriminated by a specific FISH pattern. Well-marked chromosomes were arbitrarily designated by numbers 1–7 based on their arm ratio, relative length, and FISH patterns. As a result, detailed molecular karyotypes of these species were well described (Fig. 3). Further information for the karyotypes is summarized in Tables 2 and 3.

The **P** genome was revealed by long intact chromosomes in all species of *Kengyilia*, and strong pAs1 hybridization signals in the

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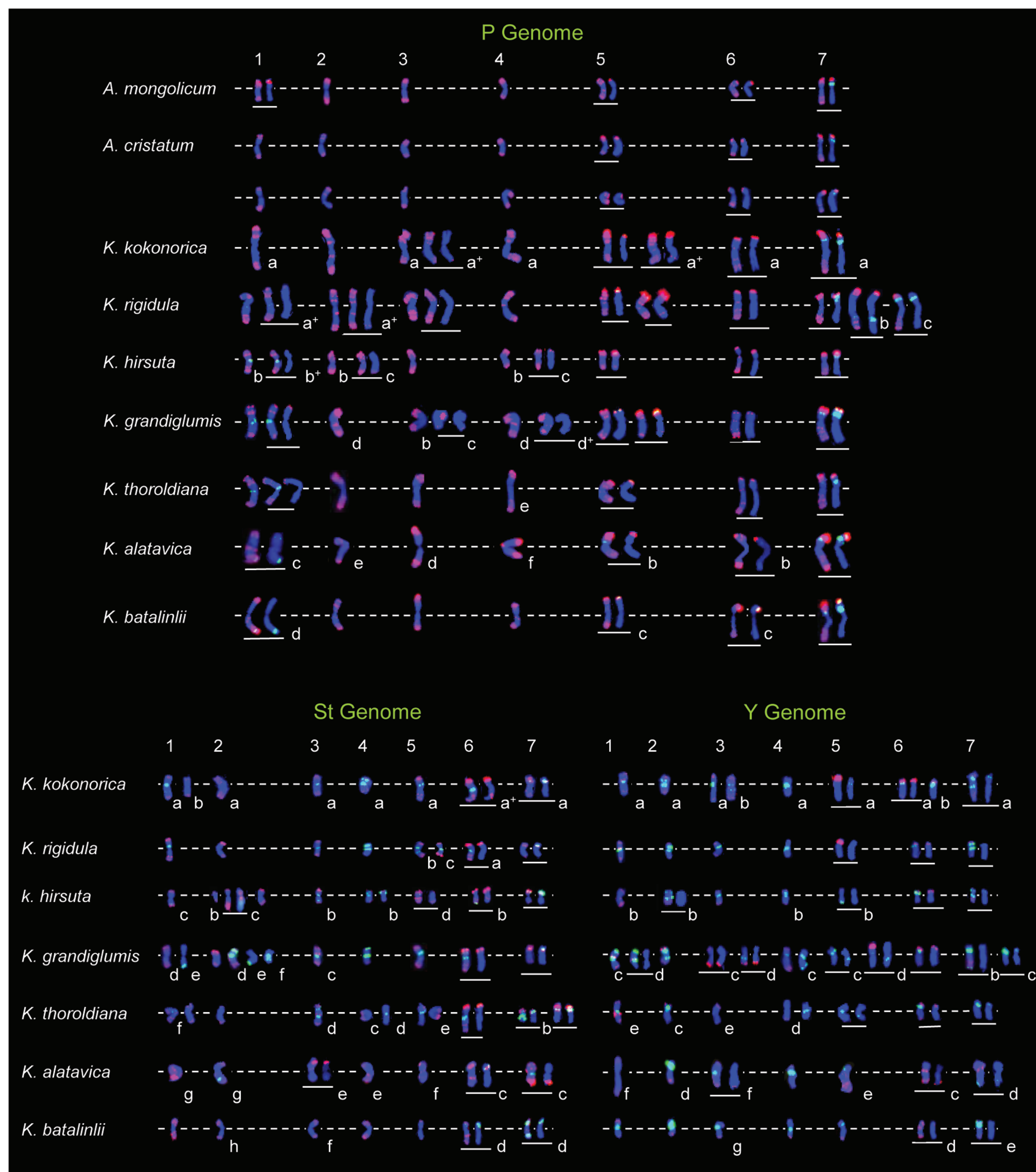
Fig. 3. Molecular karyotypes of the two species of *Agropyron* and the seven species of *Kengyilia*. The patterns of most chromosomes were characterized by pAs1 sequences (red) and $(AAG)_{10}$ (green). Underlined juxtaposed chromosomes indicate those involving 45S (red) and (or) 5S rDNA (green) sites, and translocations. Different variants are annotated by different roman letters.



subtelomeric, interstitial, or pericentric regions allowed clear discrimination between the chromosomes of the **P** genome and those of the **St** and **Y** genomes (Figs. 1 and 2). All species of *Kengyilia* and *A. mongolicum* shared the common **P**-genome karyotype formula 5”m + 2”sm, while *A. cristatum* had 5””m + 2””sm (Table 2). The FISH patterns of pAs1 in the **P** genome were well conserved on most chromosomes in the species of *Kengyilia* from Qinghai. The homoeologous chromosomes across these species could be easily

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Table 2. Kartotype features of species of *Agropyron* and *Kengyilia*.

Genome	Species	Karyotype formula	Chromosome distribution		
			45S rDNA	5S rDNA	Inversion
P	*A. mongolicum*	5”m + 2”sm	2”m (1S, 5S) + 2”sm (6S, 7S)	2”sm (1S, 7S)	—
	A. cristatum	5””m + 2””sm	2”m (5S) + 4”sm (6S, 7S)	2”sm (7S)	—
	K. kokonorica	5”m + 2”sm	2”m (3L, 5) + 2”sm (6S, 7S)	1”m (5S) + 1”sm (7S)	—
	K. rigidula	5”m + 2”sm	4”m (1S, 2L, 3L, 5) + 2”sm (6S, 7S)	1”m (5S) + 1”sm (7S)	1”sm (7)
	K. hirsuta	5”m + 2”sm	4”m (1S, 2L, 4S, 5) + 2”sm (6S, 7S)	1”m (5S) + 1”sm (7S)	—
	K. grandiglumis	5”m + 2”sm	4”m (1S, 3L, 4L, 5) + 2”sm (6S, 7S)	1”m (5S) + 1”sm (7S)	—
	K. thoroldiana	5”m + 2”sm	2”m (1S, 5S) + 2”sm (6S, 7S)	1”m (5S) + 1”sm (7S)	—
	K. alatavica	5”m + 2”sm	1”m (5S) + 2”sm (6S, 7S)	1”m (1L) + 1”sm (7S)	—
	K. batalinii	5”m + 2”sm	1”m (5S) + 2”sm (6S, 7S)	2”m (1L, 5S) + 2”sm (6S, 7S)	—
St	*K. kokonorica*	5”m + 2”sm	2”sm (6, 7S)	1”sm (7S)	—
	K. rigidula	5”m + 2”sm	2”sm (6S, 7S)	2”sm (6S, 7S)	—
	K. hirsuta	5”m + 2”sm	1”m (5L) + 2”sm (6, 7S)	1”sm (7S)	—
	K. grandiglumis	5”m + 2”sm	2”sm (6S, 7S)	2”sm (6S, 7S)	—
	K. thoroldiana	5”m + 2”sm	2”sm (6S, 7S)	2”sm (6S, 7S)	—
	K. alatavica	5”m + 2”sm	1”m (3) + 2”sm (6, 7L)	2”sm (6S, 7L)	—
	K. batalinii	5”m + 2”sm	—	2”sm (6S, 7S)	—
Y	*K. kokonorica*	5”m + 2”sm	1”sm (6S)	1”m (5S) + 1”sm (7S)	—
	K. rigidula	5”m + 2”sm	1”sm (6S)	1”m (5S) + 1”sm (7S)	—
	K. hirsuta	5”m + 2”sm	1”sm (6S)	2”m (2S, 5S) + 1”sm (7S)	—
	K. grandiglumis	5”m + 2”sm	2”m (1S, 3L) + 1”sm (6S)	1”m (5L) + 1”sm (7S)	1”sm (5)
	K. thoroldiana	5”m + 2”sm	1”sm (6S)	1”m (5L) + 1”sm (7S)	1”sm (5)
	K. alatavica	5”m + 2”sm	1”m (3L) + 1”sm (6L)	1”sm (7L)	—
	K. batalinii	5”m + 2”sm	1”sm (6S)	1”sm (7S)	—

Note: —, no chromosomes; m, metacentric; sm, submetacentric; ”, a pair of chromosomes. Chromosome numbers designated in Fig. 3 are described in parentheses.

Table 3. Chromosome variants in species of *Kengyilia*.

Genome		*K. kokonorica*	*K. rigidula*	*K. hirsuta*	*K. grandiglumis*	*K. thoroaldiana*	*K. alatavica*	*K. batalinii*	No. of variants	Total
P	1	a	a/a^{+}	b/b^{+}	b/b^{+}	b/b^{+}	c	d	6	34
	2	a	a/a^{+}	b/c	d	d	e	a	6	
	3	a/a^{+}	a/a^{+}	a	b/c	b	d	d	5	
	4	a	a	b/c	d/d^{+}	e	f	f	7	
	5	a/a^{+}	a/a^{+}	a	a/a^{+}	a	b	c	4	
	6	a	a	a	a	a	b	c	3	
	7	a	a/b/c	a	a	a	a	a	3	
St	1	a/b	a	c	d/e	d/f	g	g	7	40
	2	a	a	a/b/c	a/d/e/f	a	g	h	8	
	3	a	a	b	c	d	e	f	6	
	4	a	a	a/b	a	c/d	e	e	5	
	5	a	b/c	d	a	a/e	f	f	6	
	6	a^{+}	a	b	a	a	c	d	4	
	7	a	a	a	a	a/b	c	d	4	
Y	1	a	a	b	c/d	e	f	a	6	35
	2	a	a	b	a	c	d	d	4	
	3	a/b	a	a	c/d	e	f	g	7	
	4	a	a	b	a/c	a/d	a	a	4	
	5	a	a	b	c/d	c	e	e	5	
	6	a/b	a	a	a	a	c	d	4	
	7	a	a	a	b/c	c	d	e	5	

Note: Letters with superscript as + indicate sharing the same FISH patterns with the same letters, except additional 45S rDNA sites. The columns with shading indicate the sharing of at least one identical variant.

identified using this marker. However, the hybridization patterns of pAs1 in most of the chromosomes of the species of *Agropyron*, *K. alatavica*, and *K. batalinii* were different from those in the species of *Kengyilia* from Qinghai. One AAG site in the centromeric region of **P**-genome chromosome 1 was detected in all species of *Kengyilia* from Qinghai that was distinct from the other species, and the hybridization intensity of AAG was stronger in *K. hirsuta*, *K. grandiglumis*, and *K. thoroldiana* than in *K. kokonorica* and *K. rigidula*. Multiple 45S rDNA and 5S rDNA sites were found in the **P** genome in all species. The number of 45S rDNA sites varied from three to seven among all species of *Kengyilia* (Fig. 3; Table 2). The 45S rDNA sites on the short arms of chromosomes 5, 6, and 7 were conserved across all species of *Agropyron* and *Kengyilia*. An additional 45S rDNA site was detected on the short arms of chromosome 1 in *A. mongolicum* and a few species of *Kengyilia* from Qinghai. A few other 45S rDNA sites on 2L, 3L, 4L, and 5L were uncovered in some species of *Kengyilia* from Qinghai. The number of 5S rDNA sites varied from two to four across the tested species. All species carried a 5S rDNA site on the short arms of chromosome 7. A 5S rDNA site was present on the short arms of chromosome 5 in all species of *Kengyilia* from Qinghai. An extra 5S rDNA site on the long arms of chromosome 1 in *K. alatavica* and

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K. batalinii was prominently distinguished from the other species. Since more than one sample was tested, a few intra- or interpopulation chromosome variants were observed in the **P** genomes of the species of *Kengyilia* from Qinghai. Most of these chromosome variants were detected by the presence or absence of 45S rDNA sites. Two exceptional variants of chromosome 7 that originated from a pericentric inversion and a short arm terminal deletion were detected in a population of *K. rigidula* (Fig. 3, **P** genome 7b and 7c). Comparison of molecular karyotypes of the **P** genome between species of *Agropyron* and species of *Kengyilia* from Qinghai showed that three chromosomes (chromosomes 5, 6, and 7) shared identical or highly similar FISH patterns (Table 3). Except for chromosome 7, the chromosomes in *K. alatavica* and *K. batalinii* showed different FISH patterns from those in the *Agropyron* and species of *Kengyilia* from Qinghai (Table 3). Furthermore, genome differentiation was still observable in the species of *Kengyilia* from Qinghai. Three chromosomes in *K. hirsuta* and four chromosomes in *K. grandiglumis* and *K. thoroldiana* had different FISH patterns from the other two species of *Kengyilia* from Qinghai. The FISH patterns of two chromosomes in *K. hirsuta* were also different from those in *K. grandiglumis* and *K. thoroldiana*. The chromosomes of the **P** genome showed different numbers of variants across different species (Table 3). Chromosome 7 was the most conserved and had an identical FISH pattern in all 9 species. Chromosomes 5 and 6 were the second most conserved, with identical patterns detected in all species of *Kengyilia* from Qinghai.

The karyotype formula for the **St** genome was revealed as 5”m + 2”sm in all tested species of *Kengyilia* (Table 2). Most of the **St**-genome chromosomes displayed pAs1 hybridizations in the subtelomeric regions on both the short and long arms, and a few **St** chromosomes showed AAG signals in the subtelomeric, interstitial, or pericentric regions. The number of 45S rDNA and 5S rDNA sites varied in the **St** genome, though most were located on chromosomes 6 and 7 (Fig. 3; Table 2). An identical FISH pattern for chromosome 7 with a co-localized 45S rDNA and 5S rDNA site on the short arms was observed in all species of *Kengyilia* from Qinghai. A variant of chromosome 7 with a co-localized 45S rDNA and 5S rDNA site on the long arms was observed in *K. alatavica*, and another variant of chromosome 7 with only one 5S rDNA site present on the short arms was found in *K. batalinii*. Chromosome 6 carried at least one 45S rDNA site on the short arms in all species, except in *K. batalinii.* Additional 45S rDNA sites on chromosome 6 were found in the interstitial regions of the long arms in *K. kokonorica* and *K. hirsuta*. 5S rDNA sites on the short arms of chromosome 6 were detected in all species, except *K. kokonorica* and *K. hirsuta*. Furthermore, additional 45S rDNA sites were detected on the long arms of chromosome 5 and on the short arms of chromosome 3 in *K. hirsuta* and *K. alatavica*, respectively. Comparison of molecular karyotypes of the **St** genome showed that few identical variants were commonly shared between the species of *Kengyilia* from Qinghai and *K. alatavica* or *K. batalinii*. The number of commonly shared chromosomes in the species of *Kengyilia* from Qinghai varied from three to six. Six identical or highly similar chromosome variants were identified between *K. kokonorica* and *K. rigidula*. Five identical variants were commonly shared by *K. grandiglumis* and *K. thoroldiana*. *Kengyilia hirsuta* shared the least identical chromosomes (three) with the other species of *Kengyilia* from Qinghai (Table 3). Notably, a non-Robertsonian translocation with a chromosome segment from the **P** genome was frequently observed in the *K. hirsuta* population (Fig. 1*f3*). The chromosome variations of the **St** genome were mostly due to the presence or absence of extra pAs1, AAG, 45S rDNA, or 5S rDNA signals. Different chromosomes still showed varying degrees of intra- or interspecies differentiation. Chromosomes 6 and 7 were the most conserved, while chromosomes 1 and 2 were the most variable.

The karyotype formula 5”m + 2”sm was uncovered for the **Y** genome in all species of *Kengyilia* (Table 2). The pAS1 hybridization signals in the subtelomeric regions on both ends of most **Y**-genome chromosomes were fainter than those on **St**- and **P**-genome chromosomes. The number of **Y**-genome chromosomes showing AAG signals was generally higher than for the **St**-genome chromosomes. One 45S rDNA satellite site was detected on the ends of the short arms of chromosome 6 in all species, except *K. alatavica*, in which it was on the ends of the long arms. Additional 45S rDNA sites were detected on chromosome 3 in *K. alatavica* and on chromosomes 1 and 3 in *K. grandiglumis*. One to three 5S rDNA sites were found in the tested species. One 5S DNA site was detected on the short arms of the chromosome 7 in all species, except *K. alatavica*, in which it was detected on the long arms. One 5S rDNA site was detected in the proximal region of the short arms of chromosome 5 in all species of *Kengyilia* from Qinghai, except *K. grandiglumis* and *K. thoroldiana*, in which it was in the proximal region of the long arms. An additional 5S rDNA was detected on chromosome 2 only in *K. hirsuta* (Fig. 3; Table 2). Comparison of molecular karyotypes showed that the **Y**-genome chromosomes of *K. alatavica* and *K. batalinii* were distinct from those of the species of *Kengyilia* from Qinghai by having only one identical variant. *Kengyilia kokonorica* and *K. rigidula* shared the most conserved karyotypes, with seven identical chromosomes. *Kengyilia hirsuta*, *K. grandiglumis*, and *K. thoroldiana* were divergent from the other Qinghai species with two to three identical chromosome variants. Divergence between *K. alatavica* and *k. batalinii* was observed with only two identical chromosomes (Table 3). The chromosome variation types were much like those in the **St** genome, shown as the presence and absence of FISH probe signals. However, the 5S rDNA site in the proximal region of the long arms of chromosome 5 in *K. grandiglumis* and *K. thoroldiana* strongly suggested that there was a pericentric inversion compared with the other species of *Kengyilia* from Qinghai, in which the 5S rDNA site was located in the proximal region of the short arms (Fig. 3, Y genome). Chromosomes 2, 4, and 6 were the most conserved across species, while chromosome 3 appeared the most variable.

Discussion

The species of *Kengyilia* were suggested to be geographically differentiated in Central Asia and the Qinghai–Tibetan plateau (Zhang et al. 2005; Zeng et al. 2008*a*, 2008*b*). Phylogenetic analysis of *Pgk*1 sequences suggested that species of *Kengyilia* from Central Asia and the Qinghai–Tibetan plateau have independent origins with geographically differentiated **P**-genome donors (Fan et al. 2012). Our study showed that the molecular karyotypes of the **P** genome in *K. alatavica* and *K. batalinii* were distinct from those of the species of *Kengyilia* from Qinghai. Though differentiation of the **P** genome was observed in the species of *Kengyilia* from Qinghai, the conserved FISH patterns on most chromosomes suggest that they share a common **P**-genome donor. Differentiation of the **P** genome was still evident between *K. alatavica* and *K. batalinii*. However, some key chromosomal markers, like the 5S rDNA on the long arms of chromosome 1 that is commonly shared by both species, suggest that they share another donor of the **P** genome. Karyotype comparison between species of *Agropyron* and *Kengyilia* indicated that the molecular karyotype features of the Qinghai-originated *A. cristatum* and Inner Mongolia-originated *A. mongolicum* were closer to the **P** genome of the species of *Kengyilia* from Qinghai than that of *K. alatavica* and *K. batalinii*. Moreover, the karyotypes of the **St** and **Y** genomes in *K. alatavica* and *K. batalinii* were distinct from those of the species of *Kengyilia* from Qinghai. This implies that *K. alatavica* and *K. batalinii* possibly possess different **StY** donors from the species of *Kengyilia* from Qinghai. The karyotype differentiation of the **St** and **Y** genomes between *K. alatavica* and *K. batalinii* suggests that they possibly share different geographically differentiated **StY** donors. Our results support the hypothesis that the tested species of *Kengyilia* have independent origins in Central Asia and the Qinghai–Tibetan plateau—not only with geographically differentiated **P**-genome donors, but also with geographically differentiated **StY**-genome donors.

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The similarity of the karyotypes observed in the species of *Kengyilia* from Qinghai suggests that they are closely related. However, genome differentiation between these species could still be detected. *Kengyilia kokonorica* and *K. rigidula* had highly similar molecular karyotypes. *Kengyilia hirsuta* showed a differentiated karyotype from the others, especially in the **St** and **Y** genomes, and a unique **St-P** non-Robertsonian chromosome variant that was not observed in the other species. *Kengyilia grandiglumis* and *K. thoroldiana* showed apparent differentiation from all of the other species in their **P**, **St**, and **Y** genomes. Though differentiation between *K. grandiglumis* and *K. thoroldiana* was observed, especially in the **Y** genome, an identical chromosome inversion shared by these two species in their **Y** genomes strongly suggests a common origin. Thus, our results suggest that the five species of *Kengyilia* from Qinghai can be genetically organized into three groups: the first includes *K. kokonorica* and *K. rigidula,* the second *K. hirsuta*, and the third *K. grandiglumis* and *K. thoroldiana*.

The **P** genome was thought to be independently inherited and unable to combine with other genomes in Triticeae (Dewey 1984). In our study, all tested samples had a whole set of seven pairs of **P**-genome chromosomes. In an exceptional case, some individuals in *K. hirsuta* carried not only seven pairs of **P**-genome chromosomes, but also a non-Robertsonian translocation chromosome that consisted of most of a **St** chromosome and a small segment from the **P** genome. Translocations between **St-** and **P**-genome chromosomes could occur because of their moderately high affinity (Wang et al. 1986) and the naturally occurring **StP** genome of species of *Douglasdeweya* (Yen et al. 2005). Karyotype analysis in sympatrically distributed species with a **StHY** haplome revealed high frequency translocations between **H** and **St** or **Y** genomes (Dou et al. 2009, 2011). These results still support that the **P** genome is more “solid” than other genomes in Triticeae as suggested by Dewey (1984). 45S rDNA sites were detected in all seven chromosomes across the different species. We observed that each chromosome of the **P** genome harbored one or two 45S rDNA sites. The variation in the number of 45S rDNA sites between different species was mostly due to changes in copy number.

Chromosome rearrangements are always observed in polyploid species of Triticeae (Naranjo et al. 1987; Naranjo 1990; Liu et al. 1992; Jiang and Gill 1994). Generally, species-specific chromosome rearrangements play a key role in the speciation of polyploid plants. A theory of nucleo-cytoplasmic interaction (NCI) was hypothesized to explain the presence of species-specific translocation by Gill (1991). According to this hypothesis, a new amphiploid must pass through a bottleneck of sterility resulting from the adverse interaction between the male nuclear genome and both the nuclear and cytoplasmic genomes of the female parent. Certain bottleneck (species-specific) chromosomal changes must occur in the nuclear genome to restore fertility and nucleo-cytoplasmic compatibility. The discovery of different species-specific chromosome translocations in emmer (AABB) and timopheevi (A^tA^tGG) wheat are compatible with both the NCI hypothesis and with evidence of cytoplasmic differentiation between A^tA^tGG and AABB species (Jiang and Gill 1994). Evidence from COXII (cytochrome oxidase) intron sequences of the mitochondrial genome in species of *Kengyilia* placed the species into two clades corresponding to different maternal genomic donors (Zeng et al. 2010). Of the species used in this study, *K. hirsuta*, *K. grandiglumis*, and *K. thoroldiana* were related to species of *Agropyron*, while *K. kokonorica* and *K. rigidula* were close to *Roegneria* and *Pseudoroegneria* (Zeng et al. 2010). Our results revealed that *K. kokonorica* and *K. rigidula* share highly similar karyotypes that differ from those of *K. hirsuta*, *K. grandiglumis*, and *K. thoroldiana*. This implies that although the five species of *Kengyilia* from Qinghai share common donors in *Agropyron* and *Roegneria*, their genomic structure is strongly diverged, mostly because of their different cytoplasms. Moreover, a specific chromosome inversion was distinctly identified in *K. grandiglumis* and *K. thoroldiana*, and a **St-P** translocation was identified in some individuals in *K. hirsuta.* In field observations, the species *K. grandiglumis* and *K. thoroldiana* always have high seed-set, while *K. hirsuta* has mostly sterile individuals. According to the NCI hypothesis, the species-specific inversions and **St-P** translocation may play an important role in overcoming nucleo-cytoplasmic incompatibility and the sterility of the raw amphiploids. Chromosome variants were mostly detected by the absence or presence of FISH signals. This suggests that gene duplication and deletion may be a common occurrence in the **StYP** haplome. Only one **St-P** intergenomic translocation was identified, in some individuals of *K. hirsuta*, whereas two different inversions were identified in the **P** and **Y** genomes. Intergenomic translocation can be easily identified by GISH, whereas chromosome inversion is much more difficult to detect because of the limited chromosome markers. This implies that the actual number of inversions may be higher than the number identified. The failure to detect intergenomic recombination in *K. thoroldiana* in this study is contrary to the observation of frequent occurrence in an early study of Wang et al. (2012). This is probably due to the sample size, i.e., only one population above 4000 m of altitude was studied in this study, whereas nine populations were analyzed in Wang et al. (2012). Also, the frequent intergenomic translocations revealed in the **StHY**-genome species (Dou et al. 2009, 2011) would support that gene duplication, deletion or inversion, as well as intergenomic translocation may play an important role in speciation and adaptation of the **StPY**-genome species.

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