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RESEARCH ARTICLE

Genome constitution and evolution of *Elymus atratus* (Poaceae: Triticeae) inferred from cytogenetic and phylogenetic analysis

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Abstract

Background *Elymus atratus* (Nevski) Hand.-Mazz. is perennial hexaploid wheatgrass. It was assigned to the genus *Elymus* L. sensu stricto based on morphological characters. Its genome constitution has not been disentangled yet.

Objective To identify the genome constitution and origin of *E. atratus*.

Methods In this study, genomic in situ hybridization and fluorescence in situ hybridization, and phylogenetic analysis based on the *Acc1, DMC1* and *matK* sequences were performed.

Results Genomic in situ hybridization and fluorescence in situ hybridization results reveal that *E. atratus* 2n = 6x = 42 is composed of 14 **St** genome chromosomes, 14 **H** genome chromosomes, and 14 **Y** genome chromosomes including two **H**-**Y** type translocation chromosomes, suggesting that the genome formula of *E. atratus* is **StStYYHH**. The phylogenetic analysis based on *Acc1* and *DMC1* sequences not only shows that the **Y** genome originated in a separate diploid, but also suggests that *Pseudoroegneria* (**St**), *Hordeum* (**H**), and a diploid species with **Y** genome were the potential donors of *E. atratus*. Data from chloroplast DNA showed that the maternal donor of *E. atratus* contains the **St** genome.

Conclusion *Elymus atratus* is an allohexaploid species with **StYH** genome, which may have originated through the hybridization between an allotetraploid *Roegneria* (**StY**) species as the maternal donor and a diploid *Hordeum* (**H**) species as the paternal donor.

Keywords *Acc1* · *Campeiostachys* · *DMC1* · *matK* · In situ hybridization · Taxonomy

Introduction

Traditionally, species in Triticeae with the same genome or genome combinations were classified into the same genus (Löve 1984; Lu 1993; Yen et al. 2005; Zhang and Zhou 2007; Baum et al. 2011; Lucía et al. 2019). Existing studies suggested that *Elymus* sensu lato included combinations of seven different basic genomes: **St**, **H**, **P**, **W**, **Ns**, **Y**, and **Xm** (Baum et al. 1995; Zhang and Zhou 2007; Dong et al. 2015). Based on the genome combinations, *Elymus* s.l. was further divided into ten genera, including the *Elymus* s.s. (**StH**), *Roegneria* K. Koch (**StY**), *Douglasdeweya* C. Yen, J. L. Yang & B. R. Baum (**StP**), *Stenostachys* Turcz. (**HW**), *Hystrix* Moench (**NsXm**), *Campeiostachys* Drobov (**StYH**), *Kengyilia* C. Yen & J. L. Yang (**StYP**), *Anthosachne* Steudel (**StYW**), *Pascopyrum* Á. Löve (**StHNsXm**), and *Connorochloa* Barkworth, S. W. L. Jacobs & H. Q. Zhang (**StYWH**) (Yen and Yang 1990; Cai 1997; Yen et al. 2005, 2006, 2011, 2013; Barkworth et al. 2009; Baum et al. 2011). As cryptic

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genera, *Elymus* and *Campeiostachys* are difficult to distinguish based on their morphology. In recent years, several suspected species in *Elymus* have been identified with the **StYH** genome and were classified into the genus *Campeiostachys* (Yang et al. 2015, 2016; Tan et al. 2021, 2022). At present, the genome composition of many species in *Elymus* has not been identified.

Campeiostachys is an allohexaploid perennial genus of the Triticeae (Poaceae) with **StYH** genome, *Campeiostachys schrenkiana* (Fisch. & C. A. Mey.) Drobov as the type species (Baum et al. 2011; Yen et al. 2013). The genus was established by Drobov in 1941 and revised by Baum et al. (2011) and Yen et al. (2013), with 14 species and 13 varieties at present (Baum et al. 2011; Yen et al. 2013; Yang et al. 2015, 2016; Tan et al. 2021, 2022). The majority of the species in *Campeiostachys* are from Asia, particularly western China, but the genus extends from the Baltic to Japan (Yen et al. 2013). It has been proved that the **St** sub-genome of *Campeiostachys* originated from *Pseudoroegneria* (Nevski) Á. Löve, the **H** sub-genome originated from *Hordeum* L., and the diploid donor of the **Y** sub-genome is still under controversy and unknown (Jensen 1990; Kellogg et al. 1996; Adderley and Sun 2014). Due to the dominant effect of the genes of the **St** and **H** genomes, although *Elymus* (**StH**) and *Campeiostachys* (**StYH**) have different genome combinations, it is almost impossible to distinguish between them depending only on morphological characters (Assadi and Runemark 1995; Baum et al. 2011; Yen et al. 2013). Nevertheless, they differ in genome composition and can be easily distinguished by cytogenetic and molecular methods (Sakamoto and Muramatsu 1966; Zhang et al. 2006; Lei et al. 2016; Tang et al. 2017; Wang et al. 2019).

Genomic in situ hybridization (GISH) and genome-specific fluorescence in situ hybridization (FISH) can effectively detect the genome composition and chromosomal rearrangement of polyploid species in Triticeae (Ørgaard and Heslop-Harrison 1994; Li et al. 2001; Yu et al. 2010; Lucía et al. 2019). Dou et al. (2011) determined the genome composition of six Triticeae species using GISH. Wang et al. (2017) developed a FISH marker St_2-80 that can easily distinguish the **St** genome from other basic genomes in Triticeae. Based on GISH, Lucía et al. (2019) discovered a species in Triticeae whose genome composition is $\mathbf{E^bP}$ and established a new genus *Pauneroa* V. Lucía, E. Rico, K. Anamth.-Jon. & M. M. Mart.Ort.

Phylogenetic analysis has been a successful tool for investigating the genome composition, phylogenetic relationship, and parental donor of Triticeae species (Huang et al. 2002; Petersen and Seberg 2002; Nasernakhaei et al. 2015; Tang et al. 2017). The single- or low-copy nuclear genes are less susceptible to concerted evolution and can generate handy markers for polyploid phylogeny (Rauscher et al. 2004; Soltis et al. 2004; Sha et al. 2010). Chloroplast DNA (cpDNA) is inherited from the maternal donor in grasses, so it can provide useful DNA markers for identifying the maternal donor (Mason-Gamer et al. 2002; Liu et al. 2006; Dong et al. 2015; Wang et al. 2019). Tang et al. (2017) determined the genomic constitution of *Elymus villosus* Muhl. ex Willd, which is **StH** based on single-copy nuclear sequences. Wang et al. (2019) explored the origin of *Elytrigia lolioides* (Kar. et Kir.) Nevski is based on two single-copy nuclear genes, one mult-copy nuclear DNA region and one cpDNA region. In summary, GISH and FISH, in combination with molecular phylogenetic analyses based on single-copy nuclear genes and cpDNA regions are efficient to explore the genome constitution and origin of target species in Triticeae.

Elymus atratus (Nevski) Hand.-Mazz. is a perennial wheatgrass. Based on the morphological characteristics, it was assigned to the genus *Elymus* (Kuo 1987; Yen et al. 2013). At present, its genome composition is still unknown. In the present study, we performed GISH, FISH, and phylogenetic analyses based on two single-copy nuclear genes and one cpDNA region, aiming to: (1) confirm the genome composition of *E. atratus*; (2) investigate the origin of *E. atratus*, and provide insight into its taxonomic treatments.

Materials and methods

Plant materials

In this study, samples of *E. atratus* were collected from Sichuan, China, with accession number ZY15003. The voucher specimens of *E. atratus* were deposited in the Herbarium of Triticeae Research Institute of Sichuan Agricultural University, China (SAUTI). St_2-80 (**St** genome-specific probe) (Wang et al. 2017), the genomic DNA of *Hordeum bogdanii* Wilensky (**H**, 2n = 14) and the genomic DNA of *Roegneria ciliaris* (Trin.) Nevski (**StY**, 2n = 28) were used as probes.

E. atratus was also used for phylogenetic analyses. For the phylogenetic analyses, the *Acc1*, *DMC1*, and *matK* accessions of diploid species and polyploid species with three different genome combinations (**StY**, **StH**, **StYH**) from Triticeae were downloaded from GenBank (http://www.ncbi.nlm.nih.gov). The basic information about these sequences is listed in Tables S1, S2, and S3.

Chromosome preparation and in situ hybridization

The roots meristems were collected from adult plants, further treated with nitrous oxide at 1mpa for three hours, then fixed with 90% glacial acetic acid, and kept with 70% alcohol. The chromosome plates were prepared using drop methods (Zhang et al. 2006). The CTAB method (Doyle and Doyle 1990) was used to extract the total genomic

DNA from fresh leaves. Plasmid St_2-80 was extracted using EndoFree Maxi Plasmid kit (TANGEN BIOTECH, Beijing, China). DNA was labeled using DIG-Nick Translation Kit (Roche, Indianapolis, IN, USA). The hybridization procedure was followed as described by Han et al. (2009). The concentration ratio of the labeling probe genomic DNA and the blocking genomic DNA was 1:100 (ng/uL). Images of GISH and FISH were captured by Olympus BX61 fluorescence microscopy (Japan). Fifteen metaphase cells for *E. atratus* were observed and images were processed using Adobe Photoshop CS6.

Sequence amplification and phylogenetic analyses

The *Acc1*, *DMC1*, and *matK* genes were amplified with the primers listed in Table 1. The 50 uL reaction mixture was used for PCR amplification, containing 25 uL 1 × phanta mix buffer, 1 mM dNTP mix, 1uL DNA polymerase (Vazyme, Nanjing, China), 10 μm of each primer, 200 ng of template DNA, and distilled deionized water to the 50 uL. PCR products were cloned into the 007VS vector (TSINGKE Biological Technology, Beijing, China). Selected independent clones for sequencing by Sangon Biological Engineering and Technology Service Ltd. (Shanghai, China).

DNA sequences were confirmed through BLAST nucleotide alignment on the NCBI database. The multiple sequences were aligned using MAFFT. jModelTest 3.0 (Posada and Crandall 1998) was used to determine appropriate DNA substitution models and gamma rate heterogeneity. Phylogenetic analyses were conducted using the maximum-likelihood method in PhyML 3.0 (Guindon et al. 2009) and Bayesian inference (BI) in MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001). Statistical support for nodes in ML analysis was estimated by using 1000 fast bootstrap replicates.

Results

GISH and FISH analyses

We conducted monochrome GISH by using the **H** genome (from *H. bogdanii*) as probe and **StY** genome (from *R. ciliaris*) as block. The results showed that *E. atratus* contains 2n = 42 chromosomes (Fig. 1a, d). Fourteen of 42 chromosomes displayed strong **H** signal on the entire length (Fig. 1b, c). In addition, the two other chromosomes also showed a bright **H** hybridization signal but only in part of the long arms of the chromosomes (Fig. 1c). In contrast, 28 chromosomes displayed a **StY** signal when using the **StY** genome as probe and the **H** genome as block (Fig. 1e, f). Specifically, twenty-six chromosomes exhibited the entire chromosome signal while two chromosomes showed a partly bright hybridization signal (Fig. 1f). The results of double-color GISH are consistent with monochrome GISH (Fig. 1g–i). Thus, the genomic constitution of *E. atratus* is **StYH** with one pair of chromosomes showing translocations between **H** and **St/Y** subgenomes.

To determine the translocation type in the two translocated chromosomes, we performed GISH and FISH using the **H** genome (from *H. bogdanii*) and St_2-80 (**St** genome-specific FISH probe) as probes to distinguish **H** and **St** genomes, respectively (Fig. 2). The GISH results showed that 14 chromosomes of *E. atratus* displayed **H** signals on the entire length, and part of the long arms of two chromosomes also showed **H** genome signals (Fig. 2b, d). The FISH results showed that 14 chromosomes displayed St_2-80 signal and no **St**-type signal was observed on the two translocated chromosomes (Fig. 1c, d). It is no doubt that the translocation type in the two translocated chromosomes is between the subgenome **H** and the subgenome **Y**.

Table 1 The primers used in this study

Gene	Primer	Sequence of primer (5′–3″)	Profiles
Acc1	AccF1	CCCAATATTTATCATGAGACTTGCA	1 cycle: 5 min 95 ℃; 35 cycles: 30 s 95 ℃, 30 s 56 ℃, 2 min 30 s 68 ℃; 1 cycle: 10 min 68 ℃.
	AccF2	CAACATTTGAATGAATHCTCCACG	
DMC1	TDMC1e10F	TGCCAATTGCTGAGAGATTTG	1 cycle: 4 min 95 ℃; 35 cycles: 1 min 95 ℃, 1 min 52 ℃, 1 min 72 ℃; 1 cycle: 10 min 72 ℃.
	TDMC1e15R	AGCCACCTGTTGTAATCTGG	
matK	matK-F	CGATCTATTCATTCAATATTTC	1 cycle: 4 min 95 ℃; 35 cycles: 1 min 95 ℃, 1 min 50 ℃, 1 min 30 s 72 ℃; 1 cycle: 10 min 72 ℃.
	matK-R	TCTAGCACACGAAAGTCGAAGT	

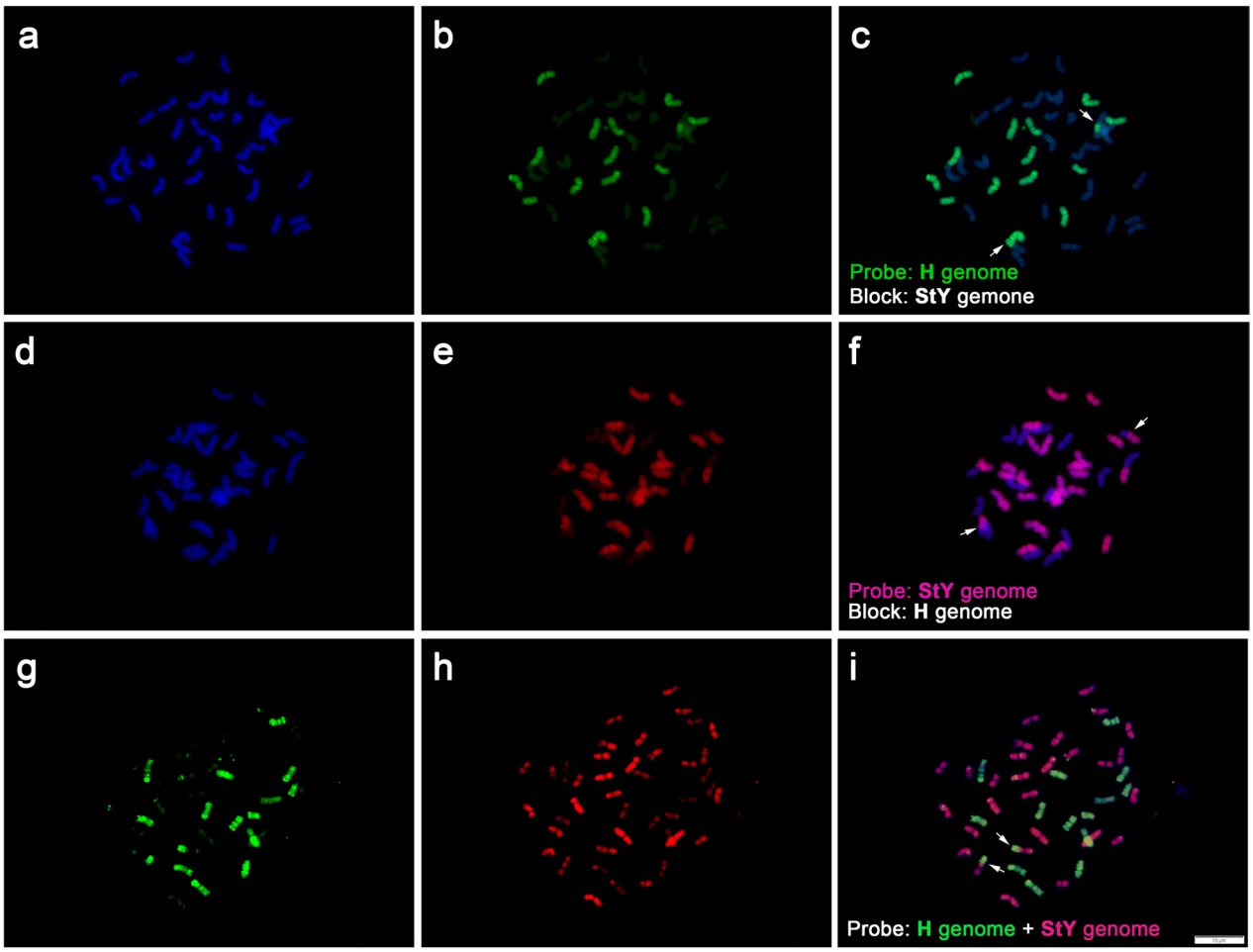

Fig. 1 GISH on somatic metaphase cells from root tips of *Elymus atratus*. **a** chromosomes of *E. atratus* counterstained by DAPI. **b**, **c** 14 chromosomes showing **H** signal when probed with **H**-genome DNA of *H. bogdanii* and blocked with **StY**-genome DNA of *R. ciliaris*, in addition, two translocation chromosomes are observed (indicated by arrows). **d** chromosomes of *E. atratus* counterstained by DAPI. **e**, **f** 28 chromosomes showing red fluorescence (**StY**) when probed with **StY**-genome DNA of *R. ciliaris* and blocked with **H**-genome DNA of *H. bogdanii*. Two translocated chromosomes are observed (indicated by arrows). **g**–**i** 14 chromosomes showing **H** signal and 28 chromosomes showing **StY** signal using **H**-genome DNA and **StY**-genome DNA as probes. Two translocated chromosomes are observed (indicated by arrows). Bar = 10 µm

Phylogenetic analyses

Phylogenetic analysis of *Acc1* gene sequences

The *Acc1* sequence length of *Elymus atratus* ranged from 1436 to 1440 bp. The *Acc1* data matrix contained 1617 characters, of which 261 were variable characters and 130 were informative. The dataset has 71 sequences for phylogenetic analysis, using the species *Bromus inermis* L. as the outgroup. A single phylogenetic tree was generated using TIM + F + I + G4 which is the best-fitted model (− Ln likelihood = 6929.715).

Both ML and BI analyses with the *Acc1* sequences of *E. atratus* were divided into three clades, namely **St**, **Y**, and **H** clades (Fig. 3). In the **St** clade, five diploid species of *Pseudoroegneria* (**St** genome donor) plus five tetraploid species of *Elymus* with the **StH** genomes, five tetraploid species of *Roegneria* with the **StY** genomes, and *E. atratus* (BS = 85%, PP = 1.00) were clustered. The **Y** clade contained *Peridictyon sanctum* (Boiss.) Nevski with the **Xp** genome, two diploid species of *Dasypyrum* (Coss. & Durieu) T. Durand with the **V** genome, five *Roegneria* species (**StY**), and *E. atratus* (BS = 96%, PP = 1.00). The **H** clade included three diploid *Hordeum* species (**H** genome donor), five *Elymus* species (**StH**), and *E. atratus* (BS = 98%, PP = 1.00). Thus, the phylogenetic analysis of *Acc1* sequences illustrated that *E. atratus* contains **St**, **Y**, and **H** sub-genomes.

Phylogenetic analysis of *DMC1* gene sequences

The *DMC1* sequence length of *E. atratus* ranged from 978 to 981 bp. The *DMC1* data matrix contained 1121 characters,

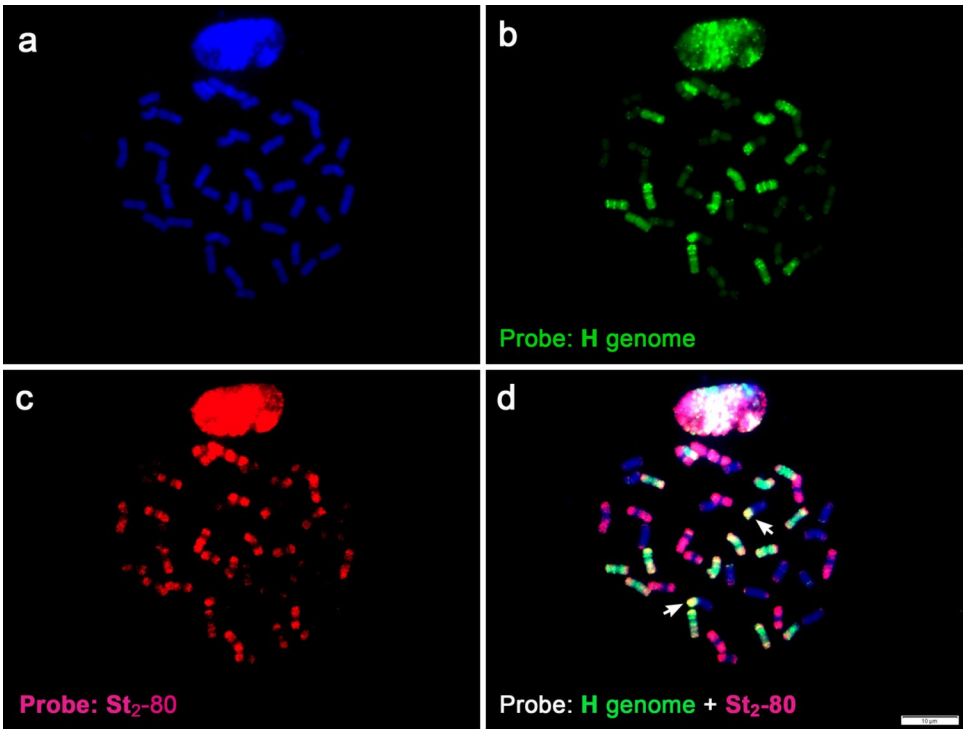

Fig. 2 GISH and FISH on somatic metaphase cells from root tips of *Elymus atratus*. **a** chromosomes of *E. atratus* counterstained by DAPI. **b H** genome DNA as probe to generate hybridization signals on chromosomes of *E. atratus* in GISH. **c** St_2-80 as probe to generate hybridization signals on chromosomes of *E. atratus* in FISH. d, 14 chromosomes of *E. atratus* showing **H** genome signals on the entire length and part of the long arms of two chromosomes also showing **H** genome signals (indicated by arrows), 14 chromosomes of *E. atratus* showing St type St_2-80 signals. Bar = 10 μm

860 of which were constant, 270 were variable, and 113 were informative. The dataset included 62 sequences for phylogenetic analysis, using the species *Bromus inermis* L. as the outgroup. We inferred a phylogenetic tree based on HKY + F + G4 which is the best-fitted model (− Ln likelihood = 4813.056).

The *DMC1* sequences from *E. atratus* were divided into three clades, named **St**, **Y**, and **H** clades, respectively (Fig. 4). The **St** clade included five diploid species in *Pseudoroegneria* (**St** genome donor), five tetraploid species in *Elymus* (**StH**), and six tetraploid species in *Roegneria* (**StY**), and *E. atratus* (BS = 96%, PP = 1.00). The **Y** clade only contained *Roegneria* (**StY**) and *E. atratus* (BS = 96%, PP = 1.00). The **H** clade contained five diploid species in *Hordeum* (**H** genome donor), five *Elymus* species (**StH**), and *E. atratus* (BS = 91%, PP = 1.00). The phylogenetic analysis of *DMC1* sequences suggested that *E. atratus* is an allopolyploid species containing **St**, **Y**, and **H** genomes.

Phylogenetic analysis of *matK* gene sequences

The *matK* sequences length of *E. atratus* was 844 bp. The *matK* data matrix contained 844 characters, 714 of which were constant, 98 were variable, and 61 were informative. This dataset contained 42 sequences for phylogenetic analysis, using the species *Bromus tectorum* L. as the outgroup. We inferred a phylogeny based on TIM + F + G4, which is the best-fitted model (− Ln likelihood = 2092.347).

The *matK* sequence from *E. atratus* was divided into two clades, named **St** + **V** + **E** + **D** and **H** (Fig. 5). The **St** + **V** + **E** + **D** clade included five diploid species in *Pseudoroegneria* (**St** genome donor), *Dasypyrum villosum* (**V** genome donor), *Thinopyrum bessarabicum* ($\mathbf{E^b}$ genome donor), *Lophopyrum elongatum* ($\mathbf{E^e}$ genome donor), one species in *Elymus* (**StH**), and 12 tetraploid species in *Roegneria* (**StY**), and *E. atratus* (BS = 63%). *E. atratus* formed a subclade with *Pseudoroegneria strigosa* (**St**), *Pseudoroegneria spicata* (**St**), and *Roegneria dura* (**StY**) (BS = 78%). The **H** clade only contained five diploid species in *Hordeum* (**H** and **I** genome donor) (BS = 100%, PP = 1.00). Other diploid species in Triticeae clustered into other clades with high support. The *matK* sequences suggest that the maternal donor of *E. atratus* contains the **St** genome.

Discussion

Genome constitution of *Elymus atratus*

According to the flora of China, *E. atratus* is a hexaploid wheatgrass and is mainly distributed in China (Sichuan, Qinghai, Gansu, Xinjiang, and Tibet). Liu (1985) conducted a karyotype analysis of *E. atratus*, which was collected in the Aba Tibetan Autonomous Prefecture of Sichuan, and the results showed that it contained 42 chromosomes. Surprisingly, cytological observations by Lu et al. (1990) not only identified the hexaploid karyotype but also reported

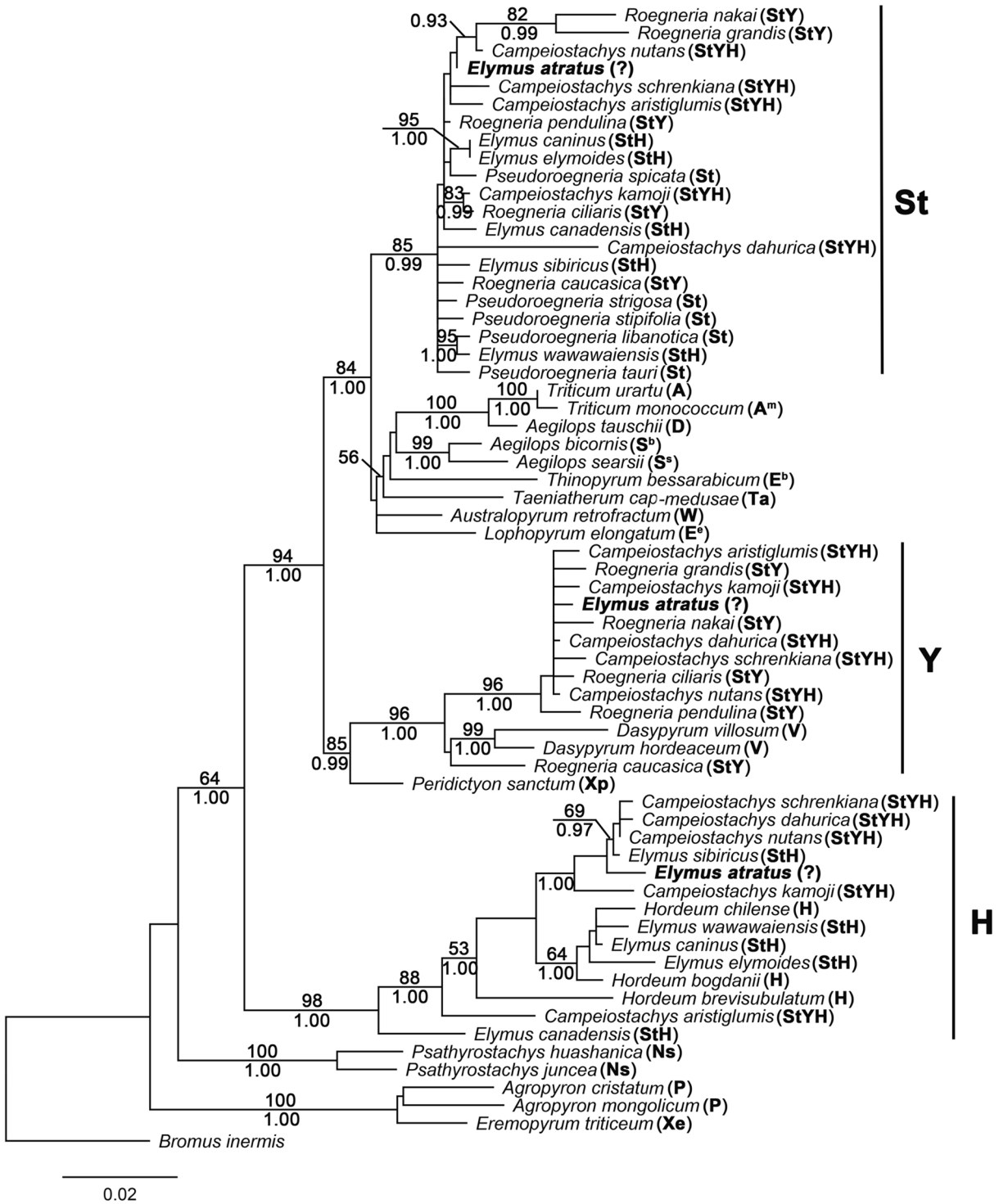

Fig. 3 Maximum likelihood tree based on *Acc1* gene sequences of *Elymus atratus* and its related species using *Bromus inermis* as outgroup. The capital letters in brackets after the species name indicate the genome composition of the species. The numbers above and below the branches indicate bootstrap values > 50% and Bayesian posterior probability values > 90%, respectively. Vertical bars indicate the subgenome that characterize each of the three main clades of the phylogeny

a tetraploid karyotype of *E. atratus*. Whether tetraploid or hexaploidy, the genome composition of *E. atratus* has not been reported in previous studies. In the present study, the results of GISH and FISH detected that *E. atratus* has 14 **St** genome chromosomes, 14 **Y** genome chromosomes, and 14 **H** genome chromosomes. The allopolyploid species

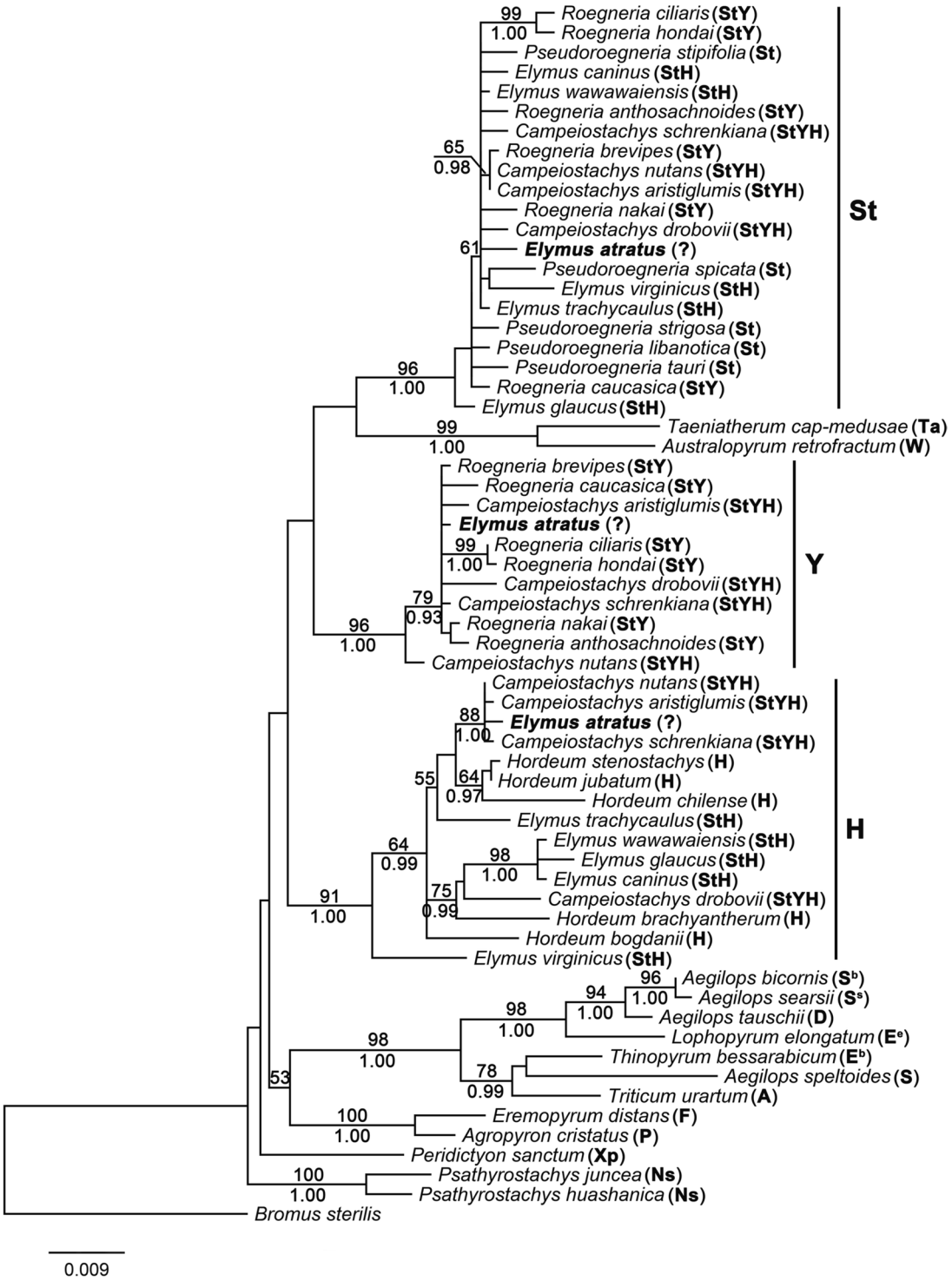

Fig. 4 Maximum likelihood tree based on *DMC1* gene sequences of *Elymus atratus* and its related species using *Bromus sterilis* as outgroup. The capital letters in brackets after the species name indicate the genome composition of the species. The numbers above and below the branches indicate bootstrap values > 50% and Bayesian posterior probability values > 90%, respectively. Vertical bars indicate the subgenome that characterize each of the three main clades of the phylogeny

in Triticeae contains a corresponding copy of each basic genome, and these copies are clustered separately with the diploid donor (Petersen et al. 2006; Lei et al. 2022). The phylogenetic analysis based on *Acc1* and *DMC1* sequences showed that *E. atratus* have **St**, **Y**, and **H** subgenome copies, suggesting that *E. atratus* is an allohexaploid species. The

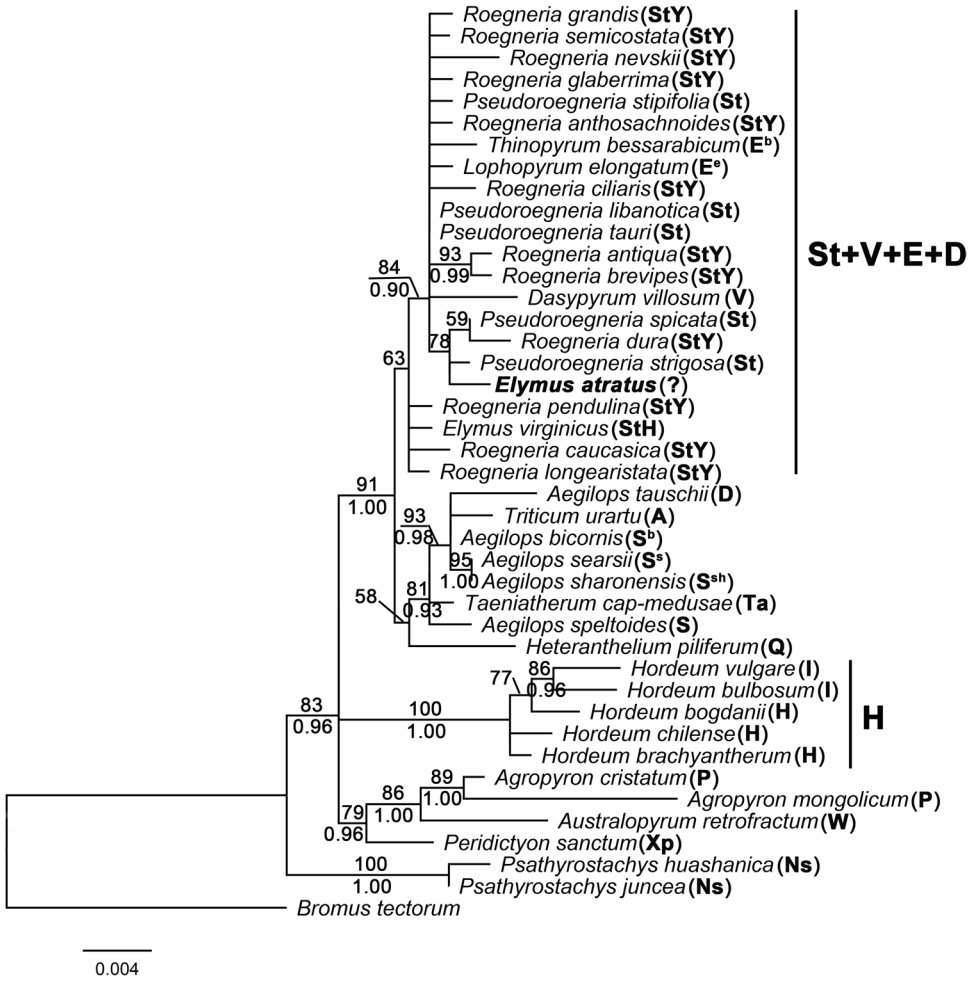

Fig. 5 Maximum likelihood tree based on *matK* gene sequences of *Elymus atratus* and its related species using *Bromus tectorum* as outgroup. The capital letters in brackets after the species name indicate the genome composition of the species. The numbers above and below the branches indicate bootstrap values > 50% and Bayesian posterior probability values > 90%, respectively. Vertical bars indicate the subgenome that characterize each of the twoe main clades of the phylogeny

results of the cytological and phylogenetic analysis proved that *E. atratus* is an allohexaploid, and the genome formula is **StStYYHH**.

Origin of the Y genome

The origin of the **Y** genome remains enigmatic. Some researchers believe that it is derived from the **St** genome, and others think its origin is from an unknown donor but has not been discovered or extinct (Jensen 1990; Kellogg et al. 1996; Mason-Gamer et al. 2005). Liu et al. (2006) based on sequences from the *ITS* nuclear DNA region concluded that the **Y** genome was gradually differentiated from the **St** genome. Okito et al. (2009) based on RAPD results also supported the **Y** genome differentiation from the **St** genome. Based on five gene sequences, Liu et al. (2022) suggested that allotetraploid species with the **StY** genome may have originated from the autotetraploid (**StSt**) of *Pseudoroegneria*. Alternatively, additional research results support the independent origin of the **Y** genome from a diploid species (Dewey 1984; Sun et al. 2008; Sun and Komatsuda 2010; Lei et al. 2022). Sun and Komatsuda (2010) inferred that the **Y** genome originated independently from a diploid species based on sequences of the *EF-G* nuclear single-copy gene and that **Y** genome was most similar to the **W** genome than to the **St** genome. Adderley and Sun (2014) based on phylogenetic analyses of sequences of the *Pgk1* single-copy gene, inferred that the **Y** genome originated independently, and the **Y** genome donor was closely related to the **V** genome and **Xp** genome. Lei et al. (2022) also supported that the **Y** and **St** genomes were of different origins and closely related to the **V** and **Xp** genomes. In our study, phylogenetic analysis results based on *Acc1* and *DMC1* sequences showed that the **St** clade and **Y** clade were separated with high support. In conclusion, we suggest that the origin of the **Y** genome and the **St** genome is independent.

Origin of *Elymus atratus*

Allopolyploids of Triticae are produced by interspecific hybridization of different genera with different sets of genomes (Sears 1954; Kimber and Alonso 1981; Dewey 1984; Heslop-Harrison 1992; Petersen et al. 2011). In the present study, the results of GISH, FISH, and phylogenetic

analyses based on the single-copy genes *Acc1* and *DMC1* confirmed that the genome composition of *E. atratus* is **StYH**. These results indicate that the parents of *E. atratus* are species with **St**, **Y**, or **H** genomes. There are several possibilities for the origin of *E. atratus*: **St** × **HY**, **Y** × **StH**, or **H** × **StY**. On the one hand, the **Y** genome originated independently from a diploid species with the **Y** genome which have not been identified yet (Torabinejad and Mueller 1993; Gao et al. 2014). On the other hand, the known genera with **Y** genome are *Roegneria* (**StY**), *Campeiostachys* (**StYH**), *Kengyilia* (**StYP**), *Anthosachne* (**StYW**), and *Connorochloa* (**StYWH**). None species with the genome combination **HY** has ever been reported. In conclusion, *E. atratus* is probably the results of the natural cross between a tetraploid *Roegneria* (**StY**) and a diploid of *Hordeum* (**H**).

The cpDNA is maternally inherited in grasses (Middleton et al. 2014). Therefore, sequences from cpDNA regions have been widely used to identify the maternal donor of allopolyploid species or genera in Triticeae (Yan et al. 2014; Dong et al. 2015; Sha et al. 2017; Wang et al. 2019). Lei et al. (2018) based on the analyses of sequences of *ndhF* and *trnH-psbA* cpDNA reginos speculated that **St** or **StY** might be the maternal donor of the species of *Campeiostachys*. In this study, phylogenetic analysis based on *matK* gene sequences revealed that *E. atratus* formed a subclade with *Pseudoroegneria strigose* (**St**), *Pseudoroegneria spicata* (**St**), and *Roegneria dura* (**StY**), this suggests that the maternal donor of *E. atratus* contains the **St** genome. In summary, we believe that *E. atratus* may have been originated from a natural cross between a tetraploid species of *Roegneria* as a maternal donor and a diploid species of *Hordeum* as a paternal donor.

Taxonomic treatment of *Elymus atratus*

The main morphological characteristics of *Elymus atratus* are as follows: leaf sheaths glabrous; leaf blades involute, often coiled; spikes flexuous and nodding, with dense spikelets; two spikelets on each rachis node, spikelets are black and purple after maturity; glumes oblong or lanceolate, apex acuminate; lemmas lanceolate, lemma awned (10–25 mm); palea equal to lemma (Yen et al. 2013). Based on the morphological characteristics, *Elymus atratus* belongs to the *Elymus* (Yen et al. 2013). But phenotype is the co-consequence of genetics and environments. Some studies have shown that there are cryptic species (such as *Roegneria panormitana* (Parl.) Nevski and *R. heterophylla* (Bornm. ex Melderis & Rech. f.) C. Yen, J. L. Yang & B. R. Baum) and cryptic genera (such as *Elymus* and *Campeiostachys*) in Triticeae (Baum et al. 2011; Yen et al. 2013). As cryptic genera, although *Elymus* and *Campeiostachys* are morphologically similar, their genome composition is different, *Elymus* contains the **StH** genome and *Campeiostachys* contains the **StYH** (Yen et al. 2013). Therefore, we can distinguish between those two genera by determining the genome composition of the species. The GISH, FISH, and phylogenetic analyses results demonstrated that the genome constitution of *Elymus atratus* is **StYH**. According to the genome classification systems in Triticeae, *E. atratus* should be transferred to the genus *Campeiostachys* and renamed *Campeiostachys atrata* (Nevski) Y. H. Zhou, H. Q. Zhang & L. Tan.

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Declarations

Conflict of interest The authors declare no conflicts of interest.

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