Phylogenetic analysis of *Elymus* (Poaceae) in western China

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ABSTRACT. *Elymus* L. is often planted in temperate and subtropical regions as forage. Species in the genus have 5 allopolyploid genomes that are found in the grass tribe Triticeae. To determine the phylogenetic relationships in *Elymus* species from western China, we estimated phylogenetic trees using sequences from the nuclear ribosomal internal transcribed spacer and non-coding chloroplast DNA sequences from 56 accessions (871 samples) of 9 polyploid *Elymus* species and 42 accessions from GenBank. Tetraploid and hexaploid *Elymus* species from western China had independent origins, and *Elymus* species from the same area or neighboring geographic regions were the most closely related. Based on the phylogenetic tree topology, the St- and Y-genomes were not derived from the same donor and Y-genome likely originated from the H-genome of *Hordeum* species, or they shared the same origin or underwent introgression. The maternal genome of tetraploid and hexaploid *Elymus* species originated from species of *Hordeum* or *Pseudoroegneria*. Additionally, *Elymus* species in western China began diverging 17-8.5 million years ago, during a period of increased aridification as a consequence of the Messinian salinity crisis. *Elymus* species adapted to drought and high salinity may have developed based on the environmental

conditions during this period. *Elymus* evolution in western China may have been affected by the uplift of the Qinghai-Tibetan Plateau (5 million years ago), when *Elymus* seeds were dispersed by gravity or wind into a newly heterogeneous habitat, resulting in isolation.

INTRODUCTION

Hybridization and polyploidization play important roles in plant evolution. Polyploidization, or chromosome doubling, can promote changes in genome size, cell size, genomic re-patterning, gene expression, retrotransposon activation, and epigenetic effects (Otto, 2007). Roughly 70% of flowering plants are polyploids (Masterson, 1994) and there is evidence that polyploid species have a better chance of survival in unstable climates (Levin, 1983). Polyploidy can occur as autopolyploidy, by doubling a single genome, or allopolyploidy, by combining divergent genomes through hybridization (Masterson, 1994).

Elymus L. includes approximately 150 allopolyploid species in the grass tribe Triticeae and is the largest and most widely distributed genus in temperate and subtropic regions (Von Bothmer and Salomon, 1994). Notably, *Elymus* species have been widely examined in plant evolution. A chromosome pairing study found that *Elymus* species contains the St, H, P, W, and Y type genomes, potentially from the following donors: *Pseudoroegneria*, *Hordeum*, *Agropyron*, *Australopyrum*, and an unknown species, respectively (Torabinejad and Mueller, 1993). The St-genome is a shared genome found in all *Elymus* species (Dewey, 1984). Most *Elymus* species are either tetraploid StH and StY or they are hexaploid StStH, StHH, StSY, StYY, StHY, StYW, and StYP (Dewey, 1984). The StY tetraploid *Elymus* species are mainly distributed in Asia (Lu and Salömon, 1992).

Previous studies have described hypotheses for reticulate evolution estimated from molecular markers, including chloroplast DNA and high-copy and low-copy nuclear genes (Mason-Gamer et al., 2010b). Chloroplast DNA is maternally inherited in angiosperms, and generally there is a single copy of chloroplast loci present in plants. However, chloroplast DNA does not always clearly distinguish organismal phylogenetic relationships (Mason-Gamer, 2013). Low-copy nuclear genes can be inconvenient because they can be difficult to amplify because of their low-copy number, making it difficult to design amplification primers, particularly given that these regions have only been characterized in a few species (Mason-Gamer et al., 2010b). In contrast, the nuclear ribosomal internal transcribed spacer region (nrITS) is a high-copy gene that is easily cloned and is widely used for phylogenentic analyses. Distinct copies of the nrITS region may convert towards one or the other parent through a process of concerted evolution, even in polyploid genomes (Mason-Gamer, 2013), and thus some nrITS sequences may be discordant with the organismal phylogeny. Nevertheless, an analysis of numerous nrITS sequences from *Elymus* species confirmed that these sequences are suitable for estimating *Elymus* phylogenentic relationships (Liu et al., 2006).

Studies examining the phylogenetic relationships in *Elymus* species have generally used 2 approaches. Early studies examined hypotheses of phylogenetic relationships based on cytological data (Lu and Salömon, 1992). Subsequently, researchers focused on determining the origin of the Y-genome; currently, there are different views regarding the diploid donor of the Y-genome based on which molecular makers or whether the same *Elymus* species are sampled (Liu et al., 2006;

Yan et al., 2011; Fan et al., 2013). Few molecular phylogenetic studies have included samples of *Elymus* species from western China.

The goals of the present study were to 1) elucidate the phylogenetic relationships of tetraploid and hexaploid *Elymus* species from western China; 2) reveal potential diploid, maternal donors using new and unreported chloroplast *trn*L-F sequences; and 3) estimate when *Elymus* species diverged in this region to test hypotheses regarding processes that may have led to speciation in the genus.

MATERIAL AND METHODS

Plant materials and DNA extraction

We collected 42 accessions (871 samples) of 9 polyploid *Elymus* species from western China from 2011-2013 and additional sequences from GenBank, including *Elymus* (StY-genome), *Pseudoroegneria* (St-genome), and *Hordeum* (H-genome) sequences. *Bromus inermis* was used as the outgroup (Liu et al., 2006) (**Table S1**).

We identified mature plants from each accession to confirm the species determination. Seeds were germinated in a greenhouse. Total genomic DNA was extracted from fresh leaf tissue using the cetyltrimethyl ammonium bromide method (Doyle and Doyle, 1987), with some modifications.

DNA amplification and sequencing

nrITS sequences were amplified by polymerase chain reaction using the primers ITS-5 (GGAAGTAAAAGTCGTAACAAGG) and ITS4 (TCCTCCGCTTATTGATGC) (Hsiao et al., 1995). Chloroplast non-coding *trn*L-F regions were amplified with rps (CGATAACGGGACATGAAG) and trnF (ATTTGAACTGGTGACACGAG) (Taberlet et al., 1991). Polymerase chain reaction was performed using a thermocycler protocol described in a previous report (Taberlet et al., 1991; Noyes and Rieseberg, 1999). Polymerase chain reaction products were cloned into the pMD 18-T vector (TaKaRa, Shiga, Japan), following the manufacturer instructions. Three to five positive colonies for each of the 56 accessions were sequenced by TaKaRa.

Data analysis

The nrITS sequences were aligned using the MAFFT 7.0 program (Katoh and Standley, 2013) and alignments were adjusted manually. Phylogenetic trees were estimated using maximum parsimony (MP) in PAUP 4.0b10 (Swofford, 2002). In MP analysis, a parsimony heuristic search used tree bisection-reconnection branch swapping and 100 random addition replicates. All characters were specified as unweighted, and the gaps were treated as missing data. Branch support for MP trees was used to assess the reliability of individual branches given the data and the model, based on 1000 bootstrap replicates.

nrITS dating analysis was implemented in BEAST 1.7.5 (Drummond and Rambaut, 2007). Because there are no reliable fossils of *Elymus* species that can be used as calibration points, we used a secondary calibration date of 29 (9-38) million years ago (MYA) based on a study that included well-described fossil calibrations (Bouchenak-Khelladi et al., 2010). The model GTR+G

was used for dating analysis according to MrModeltest 2.3 (Drummond and Rambaut, 2007). The Markov chain Monte Carlo method was run for 40,000,000 generations, sampling every 4000 generations and discarding 1000 samples as burn-in. Parameter convergence and sample sizes were assessed using Tracer 1.4.

Median-joining (MJ) network analysis was applied to display the genealogical relationships between taxa (Jakob and Blattner, 2006; Fu and Allaby, 2010). Little information related to the phylogenetic network of *Elymus* species has been published. In the present study, we determined the haplotype of sequences, excluding the outgroup, using the DnaSP 5.0 program (Librado and Rozas, 2009) and constructed a network of the *Elymus* species using the Network 4.1 program (Fluxus Technology Ltd, Clare, Suffolk, UK). Recombination was tested using the Recco program (Maydt and Lengauer, 2006) and the MJ network was analyzed using non-recombining sequences.

RESULTS AND DISCUSSION

nrITS tree

MP analysis yielded 239 equally parsimonious trees (tree length = 225; consistency index = 0.8222; retention index = 0.7379). The strict consensus tree is shown in Figure 1. In the MP tree, clade I consisted of 2 subclades, H1-2. Subclade H1 contained 15 *Elymus* sequences, including 6 *Elymus nutans*, 4 *Elymus excelsus*, 3 *Elymus tangutorum*, and 2 *Elymus dahuricus* var. *cylindricus*. Six *E. tangutorum* were resolved in subclade H2. Additionally, 3 *Elymus dolichatherus*, 1 *Elymus tibeticus*, and 1 *Pseudoroegneria strigosa* sequences were not grouped in 1 subclade in clade I.

Clade II contained 25 accessions divided into 3 subclades. Subclade T1 included 2 *E. dahuricus*, 2 *E. dahuricus* var. *cylindricus*, 1 *E. tibeticus*, 1 *Elymus antiquus*, and 1 *E. tangutorum* sequence. Five *E. dolichatherus* and 1 *E. antiquus* sequences were resolved in subclade T2. Five *Elymus sibiricus* and 1 *E. nutans* sequences made up subclade T3. In addition, 3 *Pseudoroegneria* and 1 *Elymus* sequences were not grouped in 1 subclade in clade II. Furthermore, 4 *Hordeum* sequences with good bootstrap values were monophyletic in clade III.

The MP tree results showed that there were some single clades and low bootstrap value. This phenomenon was also observed in *Elymus* StH allotetraploids in a previous study (Mason-Gamer et al., 2010a). Clade I mainly included tetraploid *Elymus* species and clade II mainly contained hexaploid *Elymus* species (Figure 1). This indicates that tetraploid and hexaploid *Elymus* species have an independent origin.

Triticeae is widely distributed worldwide, and botanists have long been interested in how its species are related to one another. Early studies in Triticeae evolution focused on morphological characters, but it is difficult to recognize Triticeae species based on morphology. The development of molecular biology enabled to studies of the genetic diversity of Triticeae (Mason-Gamer et al., 2010b; Sha et al., 2010; Sun, 2014). Recent studies have focused on the origin and constitution of the *Elymus* genome (Sun, 2014). However, little is known regarding the genetic relationships between *Elymus* species in China.

Previously, most cytological studies of the StY-genome in *Elymus* species indicated that chromosome pairing in hybrids decreased if the hybrid parent was from a geographically distant location (Lu and Salömon, 1992). The *Elymus* species in clade I were mainly collected in Gansu Province and clade II contained *Elymus* species from the Qinghai-Tibetan Plateau, suggesting that there is a geographical component to the tree topology. The different allopolyploid types of
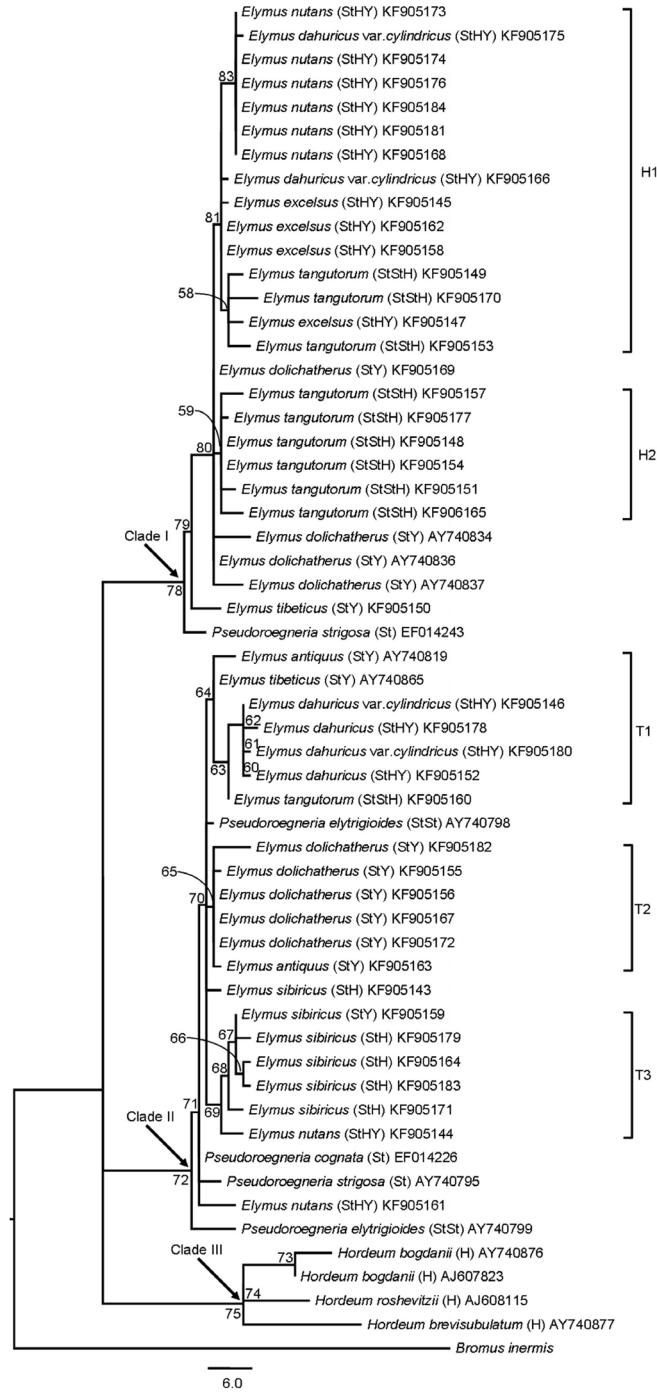
Figure 1. Maximum parsimony (MP) strict consensus tree based on nrITS sequences. Branches with bootstrap values >50% are shown. Three major clades and many minor clades are described in the text.

Elymus species may each represent distinct evolutionary lineages. For example, hexaploid *Elymus* species [except for *E. dolichatherus* (KF905169, AY740834, AY740836, and AY740837) and *E. tibeticus* (KF905150)] were in clade I, and tetraploid *Elymus* species (except for subclade T1) were in clade II. Species sharing similar morphological characters did not resolve as closely related in our trees. *E. dolichatherus* (AY740834, AY740836, and AY740837) from Sichuan Province and *E. dolichatherus* (KF905169) contained some nrITS copies in clade I, but collections (KF905155, KF905156, KF905167, and KF905172) from the Qinghai-Tibetan plateau were in clade II. Similar results were observed in *Leymus* based on nrITS sequences (Sha et al., 2008). Their results showed that species from the same areas or neighboring geographic regions were closely related to each other *Leymus* species.

There have been several other studies examining the phylogenetic relationships between polyploid *Elymus* species and their putative diploid ancestors. North American *Elymus* allotetraploids include St- (*Pseudoroegneria*) and H (*Hordeum*)-genomic components (Mason-Gamer, 2001, 2013). Phylogenetic and cytogenetic analyses of Asian *Elymus* species showed that the putative parental genomes were from *Pseudoroegneria* (St-genome), *Hordeum* (H-genome), *Agropyron* (P-genome), *Australopyrum* (W-genome), and an unknown species (Y-genome) (Sun et al., 2008). However, in this study, *Elymus* species only contained St-, H-, and Y-genomes based on cytological evidence.

Liu et al. (2006) suggested that the *Elymus* Y-genome evolved through gradual differentiation from *Pseudoroegneria* (St-genome). Our results on *Elymus* from western China do not support the results of Liu et al. (2006). *Pseudoroegneria* and the StY *Elymus* genome did not resolve as closely related (Figure 1). In addition, studies on the single copy nuclear β-amylase gene (Mason-Gamer et al., 2005), DNA polymerase subunit II (*RPB2*) sequences (Sun et al., 2008), and elongation factor G (*EF-G*) sequence data (Yan et al., 2011) have suggested that the *Elymus* Y-genome originated in an independent Y diploid species that is now extinct or undiscovered. However, Fan et al. (2013) suggested that the *Elymus* Y-genome is closely related to the *Peridictyon sanctum* (Xp-genome) based on single-copy nuclear acetyl-coa carboxylase-1 (*Acc1*) and phosphoglycerate kinase -1 (*Pgk1*) gene sequences. Therefore, the accumulated evidence suggests that the *Elymus* Y-genome originated in a different species or in multiple species. Unless a similar genome is identified in another grass, the donor of the Y-genome may never be identified (Mason-Gamer et al., 2010a; Mason-Gamer, 2013).

Interestingly, *Hordeum* species from the Xinjiang and Sichuan provinces in China are resolved in clade III, with no closely related *Elymus* species. This indicates that the *Elymus* H-genome from western China could not have originated in diploid *Hordeum* species from Xinjiang and Sichuan provinces in China. Additionally, some studies (Helfgott and Mason-Gamer, 2004; Mason-Gamer, 2013) showed that North American *Hordeum* species were the diploid progenitors of the North American and Eurasian *Elymus* species. Other studies (Sun and Komatsuda, 2010; Fan et al., 2013) showed that the *Hordeum* H-genome from western China may have been a donor to *Elymus*, such as *Hordeum bogdanii*.

Chloroplast DNA tree

Parsimony analysis of *trn*L-F sequences in this study randomly yielded 317 equally parsimonious trees, with a tree length of 310 (consistency index = 0.9000, rescaled consistency index = 0.7939). The *trn*L-F phylogenetic relationships between *Elymus* and potential donor species, based on the MP method, are shown in Figure 2.
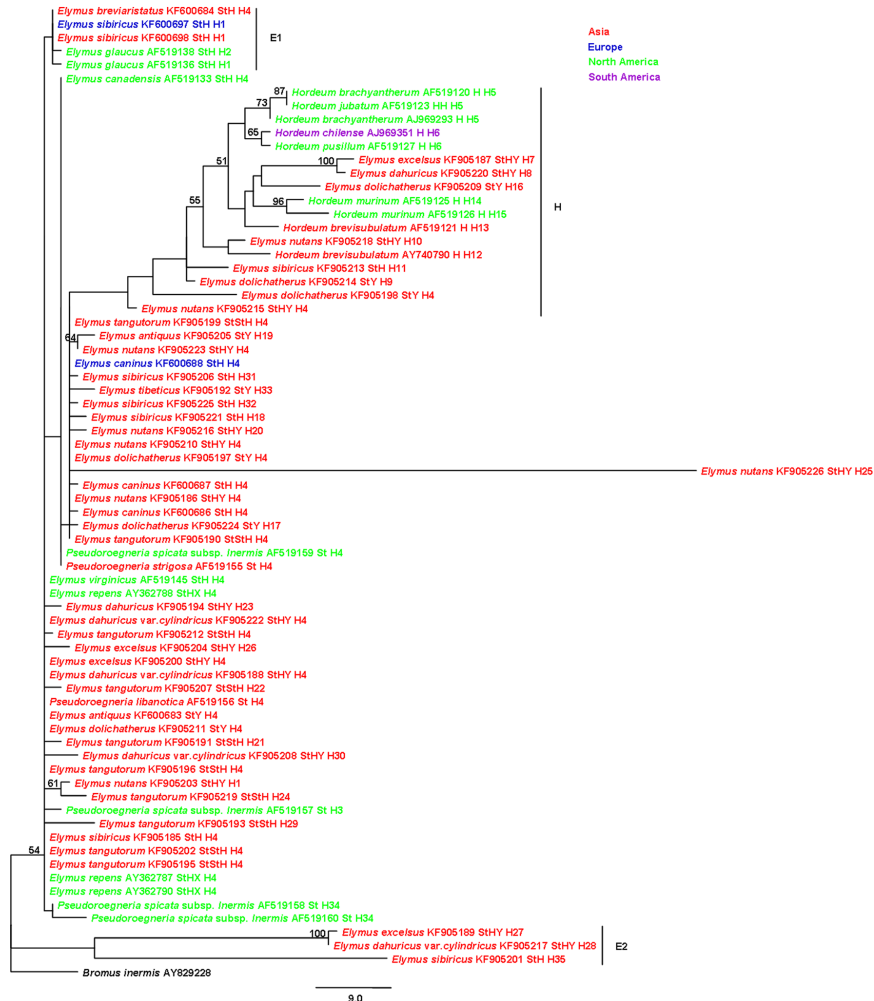
Elymus breviaristatus KF600684 StH H4
Elymus sibiricus KF600697 StH H1
Elymus sibiricus KF600698 StH H1
Elymus glaucus AF519138 StH H2
Elymus glaucus AF519136 StH H1
Elymus canadensis AF519133 StH H4

Asia
Europe
North America
South America

Hordeum brachyantherum AF519120 H H5
Hordeum jubatum AF519123 HH H5
Hordeum brachyantherum AJ969293 H H5
Hordeum chilense AJ969351 H H6
Hordeum pusillum AF519127 H H6
Elymus excelsus KF905187 StHY H7
Elymus dahuricus KF905220 StHY H8
Elymus dolichatherus KF905209 StY H16
Hordeum murinum AF519125 H H14
Hordeum murinum AF519126 H H15
Hordeum brevisubulatum AF519121 H H13
Elymus nutans KF905218 StHY H10
Hordeum brevisubulatum AY740790 H H12
Elymus sibiricus KF905213 StH H11
Elymus dolichatherus KF905214 StY H9
Elymus dolichatherus KF905198 StY H4
Elymus nutans KF905215 StHY H4
Elymus tangutorum KF905199 StStH H4
Elymus antiquus KF905205 StY H19
Elymus nutans KF905223 StHY H4
Elymus caninus KF600688 StH H4
Elymus sibiricus KF905206 StH H31
Elymus tibeticus KF905192 StY H33
Elymus sibiricus KF905225 StH H32
Elymus sibiricus KF905221 StH H18
Elymus nutans KF905216 StHY H20
Elymus nutans KF905210 StY H4
Elymus dolichatherus KF905197 StY H4
Elymus nutans KF905226 StHY H25
Elymus caninus KF600687 StH H4
Elymus nutans KF905186 StH H4
Elymus caninus KF600686 StH H4
Elymus dolichatherus KF905224 StY H17
Elymus tangutorum KF905190 StStH H4
Pseudoroegneria spicata subsp. *Inermis* AF519159 St H4
Pseudoroegneria strigosa AF519155 St H4
Elymus virginicus AF519145 StH H4
Elymus repens AY362788 StHX H4
Elymus dahuricus KF905194 StHY H23
Elymus dahuricus var.*cylindricus* KF905222 StHY H4
Elymus tangutorum KF905212 StStH H4
Elymus excelsus KF905204 StHY H26
Elymus excelsus KF905200 StHY H4
Elymus dahuricus var.*cylindricus* KF905188 StHY H4
Elymus tangutorum KF905207 StStH H22
Pseudoroegneria libanotica AF519156 St H4
Elymus antiquus KF600683 StY H4
Elymus dolichatherus KF905211 StY H4
Elymus tangutorum KF905191 StStH H21
Elymus dahuricus var.*cylindricus* KF905208 StHY H30
Elymus tangutorum KF905196 StStH H4
Elymus nutans KF905203 StHY H1
Elymus tangutorum KF905219 StStH H24
Pseudoroegneria spicata subsp. *Inermis* AF519157 St H3
Elymus tangutorum KF905193 StStH H29
Elymus sibiricus KF905185 StH H4
Elymus tangutorum KF905202 StStH H4
Elymus tangutorum KF905195 StStH H4
Elymus repens AY362787 StHX H4
Elymus repens AY362790 StHX H4
Pseudoroegneria spicata subsp. *Inermis* AF519158 St H34
Pseudoroegneria spicata subsp. *Inermis* AF519160 St H34
Elymus excelsus KF905189 StHY H27
Elymus dahuricus var.*cylindricus* KF905217 StHY H28
Elymus sibiricus KF905201 StH H35
Bromus inermis AY829228

E1
H
E2
87
73
65
51
55
96
100
64
61
54
100
9.0

Figure 2. Strict consensus tree from maximum-parsimony (MP) analysis based on *trn*L-F sequences. Numbers with bootstrap values >50% are shown. Three major clades and many minor clades are described in the text.

Compared with nuclear and chloroplast genes within phylogenetic relationships, the support provided by chloroplast genes is too weak to convincingly indicate actual polyphyletic origins (Mason-Gamer, 2013). However, chloroplast genes are inherited maternally, so the female parent of plants was recognized based on chloroplast gene sequence. In the present study, most bootstrap values on the MP phylogenetic trees were lower than 50%, and only 3 clades were recognized, including clade E1, H, and E2 (Figure 2). Clade E1 was weakly supported and contained 1 *Elymus breviaristatus* (KF600684) and 1 *E. sibiricus* (KF600698) from Asia, 1 *E. sibiricus* (KF600697) from Europe, and 2 *Elymus glaucus* (AF519136 and AF519138) from North America. Clade E2 included 3 Asian *Elymus* with stronger support values, *E. excelsus* (KF905189), *E. dahuricus* var. *cylindricus* (KF905217), and *E. sibiricus* (KF905201). Notably, 8 *Elymus* species from Asia and 8 *Hordeum* species from the Americas (7 from North America and 1 from South America) were distributed

in clade H, with well-supported values. Our analysis of the phylogenetic relationships in clade H showed that *E. excelsus* (KF905187, StHY-genome), *E. dahuricus* (KF905220, StHY-genome), and *E. dolichatherus* (KF905209, StY-genome) clustered into a subclade with *Hordeum murinum* (AF519125 and AF519126, H-genome), while *E. nutans* (KF905218, StHY-genome) and *Hordeum brevisubulatum* (AY740790, H-genome) were grouped into another subclade. This indicated that either *Hordeum* is the maternal genome donor to *Elymus* species or that there was gene flow from *Hordeum* to *Elymus* species. Previous studies showed that the female parent of North American tetraploid *Elymus* species (StH-genome) originated from North American *Pseudoroegneria* species (Mason-Gamer and Orme, 2002; Sun, 2002), indicating that the female parent of *Elymus* species from North America and Asia have different origins. Interestingly, *E. nutans* (KF905218, StHY-genome) and *H. brevisubutatum* (AY740790, H-genome) species were from Asia, while *Elymus* species in the former group were from Asia, and *Hordeum* species were from North America and Asia. In this study, we found that *Elymus* species with the Y-genome may have originated from the Qing-Tibet Plateau approximately 17-8.5 MYA. In addition, *Hordeum* species originated in the Old World approximately 12 MYA (Blatter, 2006), and New World *Hordeum* species have persisted for at least 3-4 MYA (Jakob and Blattner, 2006). A major reason why *Hordeum* species could have dispersed between the Old and New Worlds is the emergence of the Bering Land Bridge during the Pleistocene.

Moreover, *E. dolichatherus* (KF905209, StY-genome) did not contain the H-genome, yet it clustered in a clade with *Hordeum* species. It remains unclear whether the Y- and H- genomes share the same origin. As described above, several hypotheses have been suggested to explain the origin of the Y-genome. Our results were not consistent with these hypotheses. However, our results indicated that the Y- and H-genomes may have a common origin. However, Mason-Gamer (2013) suggested that the Y-genome of Asian *Elymus* does not fit into an existing genus, and instead may represent a single evolutionary lineage with subsequent introgression.

Statistical MJ network

Phylogenetic relationships reflect the hierarchical patterns of ancestry among different species, whereas networks are generally used to reflect ancestral and derived relationships (Posada and Crandall, 2001). To assess the maternal origins and biogeographical patterns of *Elymus* species, we constructed an MJ network of *Elymus* and its potential diploid donor (Figure 3). In the network, each circle indicated 1 haplotype, with the circle size proportional to the number of isolates with that haplotype, and median vectors indicating missing intermediates of unsampled nodes inferred by MJ network analysis. Different colors indicate different species; for example, yellow, gray, and green indicate *Elymus*, *Horduem*, and *Pseudoroegneria* species, respectively (Figure 3). The MJ network described the genealogical relationships among 35 haplotypes from 66 species and 7 missing intermediates (Figure 3). Among them, 5 haplotypes contained multiple species sequences: H1 included 5 Asian *Elymus* species; H4 included 26 Asian *Elymus* species, 2 Asian and 1 North American *Pseudoroegneria* species; H5 included 3 North American *Hordeum* species; H6 included 2 *Hordeum* species from North America and South America; and H34 included 2 North American *Pseudoroegneria* species. Other haplotypes included only 1 species (**Table S1**).

The network showed that *Elymus* species were grouped into 2 basic clusters of haplotypes, clusters 1 and 2. Compared to the *trn*L-F phylogenetic trees (Figure 2), cluster 2 containing *Elymus* and *Hordeum* species was consistent with clade H, except for the presence of *E. dolichatherus*

(KF905198) and *E. nutans* (KF905215) in phylogenetic trees. Therefore, the results of cluster 2 not only confirmed that the topology of the *trn*L-F phylogenetic trees was accurate, but also demonstrated that *Elymus* was the maternal donor to North American *Hordeum* species. Cluster 1 contained 20 polyloid *Elymus* haplotypes, 2 diploid *Pseudoroegneria* haplotypes, 1 nested haplotype, and 3 missing intermediates. Remarkably, *Elymus* and *Pseudoroegneria* species constituted the nested haplotype H4, indicating a contradiction between the results of *trn*L-F phylogenetic trees and the MJ network. In *trn*L-F phylogenetic analysis, *Elymus* and *Pseudoroegneria* species were not grouped into 1 clade; instead, most terminal branch lengths on phylogenetic trees were short. As described above, however, the MJ network described the genealogical relationship, and Mason-Gamer and Orme (2002) found that North American *Pseudoroegneria* species was the maternal genome donor to North American *Elymus* species. In conclusion, our results indicated that the Y-genome in Asian *Elymus* species likely had 2 patterns of maternal origin, with the first from *Hordeum* species and the second from *Pseudoroegneria* species.
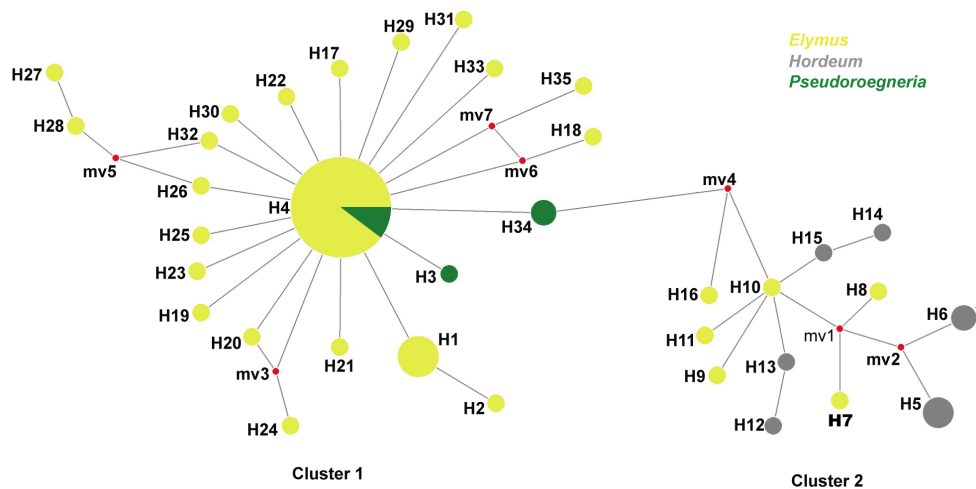

Figure 3. Median-joining (MJ) networks of *trn*L-F haplotypes from *Elymus*, *Hordeum*, and *Pseudoroegneria* species. Each circle indicated 1 haplotype, with circle size being proportional to the number of isolates with that haplotype, and median vectors indicating missing intermediates of unsampled nodes inferred by MJ network analysis. Yellow, gray, and green indicate *Elymus*, *Horduem*, and *Pseudoroegneria* species, respectively.

Dating analysis

Age estimates for the *Elymus* dataset found that the clades diverged 17-8.5 MYA. Sampled tetraploid *Elymus* species diverged approximately 17 MYA and sampled hexaploid *Elymus* species diverged approximately 8.5 MYA (Figure 4).

We estimated that *Elymus* species in western China diverged between 17-8.5 MYA. The Miocene (23-5.3 MYA) was a period of increasing aridification as a consequence of the Messinian salinity crisis (Wright and Cita, 1979). *Elymus* species may have adapted to drought and high salinity in the harsh environment during this period (Sasanuma et al., 2002). We hypothesize that selection for drought and salinity tolerance may have been the driving force for the divergence of *Elymus* species in western China, enabling them to withstand drought and high salinity.
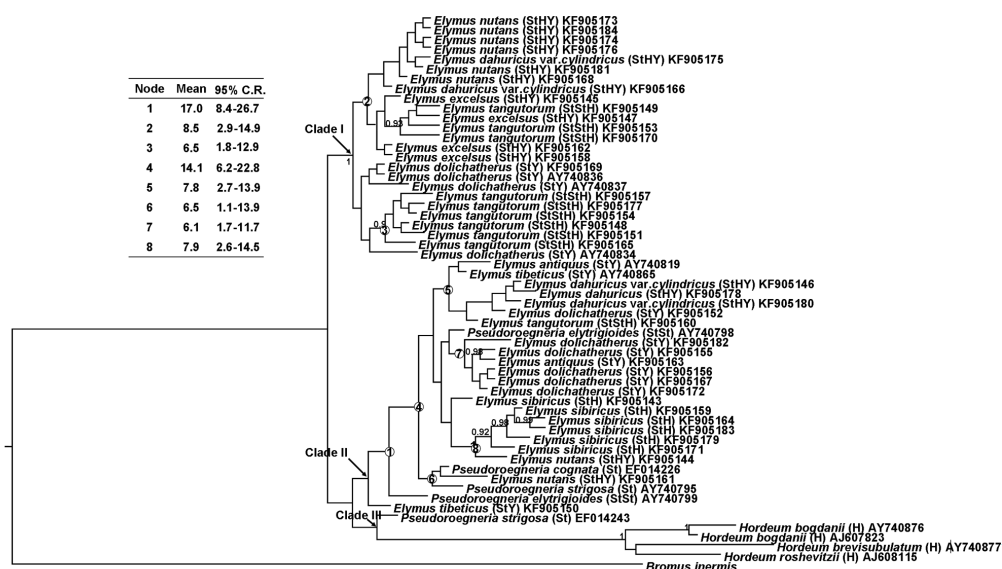
Figure 4. Bayesian analysis strict consensus tree based on nrITS sequences. Branches with posterior probabilities >0.9 are shown. Table provides the estimated divergence dates for nodes 1–8. Ages of nodes with 95% confidence ranges were obtained from BEAST molecular dating analysis. The CR ranges represent 95% highest posterior density values for nodes that received >0.5 posterior probabilities.

Central Asia is considered to be the center of origin of *Elymus* and the Y-genome is thought to have originated in Central Asia or the Himalayan region (Lu and Salömon, 1992; Torabinejad and Mueller, 1993). Geologic studies have shown that the Qinghai-Tibetan Plateau and adjacent areas were formed during large-scale uplifting in the Pliocene and during climate oscillations during the Quaternary (5 MYA) (Li and Pan, 2002). Nine *Elymus* species in this study are located mainly in Asia, particularly China (Lu and Salömon, 1992). Our dated tree indicated that the divergence times of these nodes (8) were more than 6 MYA (Figure 4), implying that these *Elymus* species already existed before uplifting of the Qinghai-Tibetan Plateau. Therefore, we suggest that 9 *Elymus* species in this study originated from the Qinghai-Tibetan Plateau. During the uplift of the Qinghai-Tibetan Plateau, *Elymus* seeds were dispersed by gravity or wind action into a newly heterogeneous habitat and experienced isolation.

Conflicts of interests

The authors declare no conflict of interest.

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Supplementary material

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