Aliso: A Journal of Systematic and Evolutionary Botany

Volume 23 | Issue 1 Article 30

2007

Allopolyploids of the Genus Elymus (Triticeae, Poaceae): a Phylogenetic Perspective

Roberta J. Mason-Gamer
University of Illinois, Chicago

Aliso 23, pp. 372–379

ALLOPOLYPLOIDS OF THE GENUS *ELYMUS* (TRITICEAE, POACEAE): A PHYLOGENETIC PERSPECTIVE

ROBERTA J. MASON-GAMER

University of Illinois at Chicago, Department of Biological Sciences, MC 066, 845 West Taylor Street, Chicago, Illinois 60607, USA
(robie@uic.edu)

ABSTRACT

The wheat tribe, Triticeae, includes many genomically distinct polyploid taxa. *Elymus* is an entirely allopolyploid genus, with all species containing the **St** genome of *Pseudoroegneria.* The **St** genome may be combined with one or more distinct genomes representing multiple, diverse diploid donors from throughout the tribe. This study includes a simultaneous phylogenetic analysis of new and previously published data from several distinct *Elymus* groups, including North American and Eurasian **StStHH** tetraploids, in which the **H** genome is derived from *Hordeum,* Eurasian **StStYY** tetraploids, in which the **Y** genome is derived from an unknown donor, and a putative **StStStStHH** hexaploid. *Elymus* species were analyzed with a broad sample of diploid genera from within the tribe using a combination of molecular data from the chloroplast and the nuclear genomes. The data confirm the genomic constitution of the **StStHH** and **StStYY** tetraploids, but do not provide additional information on the identity of the **Y**-genome donor. The genomic diversity in the hexaploid is greater than expected, inconsistent with the hypothesis of an **StStStStHH** genome complement.

Key words: *Elymus,* Poaceae, polyploidy, reticulation, Triticeae, wheat tribe.

INTRODUCTION

The wheat tribe, Triticeae (Pooideae, Poaceae), is widely known for its economic importance. This monophyletic tribe includes three major grain crops—wheat, barley, and rye—along with several important forage grasses and numerous weedy, invasive species. From an evolutionary standpoint, the tribe's complex reticulate history has been of interest for many years. At the diploid level, conflict among gene trees, especially between those based on chloroplast and nuclear DNA data (Mason-Gamer and Kellogg 1996*a*), suggests a history of gene exchange, lineage sorting, or a combination of both (Kellogg et al. 1996). Furthermore, polyploidy is common in the tribe; about 75% of the species with known chromosome numbers are polyploid (Löve 1984).

Allopolyploidy represents a reticulate process by which distinct genomes are united in a single nucleus. While there are problems with placing reticulate taxa within the bifurcating trees that are obtained using many methods of analysis (e.g., Hull 1979; Cronquist 1987; McDade 1992, 1998), studies of individual gene trees often allow these problems to be circumvented. Thus, molecular phylogenetic data have recently revealed the reticulate histories of several polyploid species or groups, for example: *Gossypium* L. (e.g., Cronn et al. 1996, 2003; Seelanan et al. 1997; Small and Wendel 2000), *Geum* L. (Smedmark et al. 2003), *Glycine* Willd. (e.g., Doyle et al. 2002; Rauscher et al. 2002), *Oxalis* L. (Emshwiller and Doyle 1998, 2002), *Oryza* L. (Ge et al. 1999), and *Paeonia* L. (e.g., Sang and Zhang 1999).

Within Triticeae, *Elymus* L. is a genomically heterogeneous polyploid group of about 140 species (Löve 1984). According to its genomic definition, *Elymus* comprises allopolyploid species with at least one set of chromosomes derived from *Pseudoroegneria* (Nevski) Á. Löve (genome designation **St**). The **St** genome can also be found in both diploid and autotetraploid species, which are classified as *Pseudoroegneria* (Dewey 1984). In *Elymus,* the **St** genome can be combined with genomes from one or more Triticeae genera, including *Hordeum* L. (genome designation **H**), *Agropyron* Gaertn. (**P**), *Australopyrum* (Tzvelev) Á. Löve (**W**), and an unknown donor (**Y**), in various allopolyploid combinations including **StStHH**, **StStYY**, **StStHHHH**, **StStStStHH**, **StStYY**, **StStStStYY**, **StStYYYY**, **StStHHYY**, **StStYYWW**, and **StStYYPP** (e.g., Dewey 1967, 1968, 1970*b*, 1974, 1984; Jensen 1990, 1993, 1996; Salomon and Lu 1992, 1994; Lu and von Bothmer 1993; Lu et al. 1995). Other **St**-containing allopolyploids include *Pascopyrum smithii* (Rydb.) Barkworth & D. R. Dewey, which combines the *Pseudoroegneria* and *Hordeum* genomes with the **Ns** genome of *Psathyrostachys* Nevski in an **StStHHNsNsNsNs** octoploid configuration (Dewey 1975), and *Thinopyrum* Á. Löve, some species of which are hypothesized to combine the **St** genome with the **E** and/or **J** genomes usually considered characteristic of *Thinopyrum* (e.g., Liu and Wang 1993; Zhang et al. 1996; Chen et al. 1998). Thus, the **St** genome, probably more than any other in Triticeae, plays an important role in the complex reticulate allopolyploid patterns that characterize the tribe.

This overview focuses on a subset of species from the genomically heterogeneous *Elymus,* and presents molecular phylogenetic data that address their relationships to the diploid members of the tribe. These species include (1) North American tetraploid species with presumed **StStHH** genome configurations (e.g., Dewey 1982, 1983*a*, *b,* 1984; Mason-Gamer 2001; Mason-Gamer et al. 2002; Helfgott and Mason-Gamer 2004), (2) Eurasian tetraploid species, with presumed **StStHH** or **StStYY** genome configurations (e.g., Lu and von Bothmer 1990, 1993; Salomon and Lu 1992, 1994; Lu 1993; Lu et al. 1995), and (3) a Eurasian hexaploid spe-

cies (*E. repens* L.), with a presumed **StStStStHH** genomic configuration (Cauderon and Saigne 1961; Dewey 1970*a*, 1976; Ørgaard and Anamthawat-Jónsson 2001). These genomically diverse allopolyploids are analyzed together with a broad sample of diploid genera from throughout the tribe, using three sources of chloroplast DNA (cpDNA) data, and sequences from the single-copy nuclear gene encoding granule-bound starch synthase (GBSSI).

MATERIALS AND METHODS

Both of the molecular data sets include a combination of new and previously published data. Specimens corresponding to the new data are currently in the possession of the author, and herbarium vouchers will be permanently deposited upon the completion of the ongoing study. Information about the previously published sequence data and the plants from which they were derived can be found in the corresponding publications, cited below.

The cpDNA data set includes restriction sites, sequences from *trn*T, *trn*L, and *trn*F genes and their intergenic spacers, and sequences of the *rpo*A gene. The existing restriction site data set (Mason-Gamer and Kellogg 1996*b*) includes most of the monogenomic genera and three species of *Elymus* (*E. lanceolatus* (Scribn. & J. G. Sm.) Gould, *E. repens,* and *E. ciliaris* (Trin.) Tzvelev); no new restriction site data were added for the present analysis. The existing tRNA/spacer and *rpo*A data sets include most of the monogenomic genera (Mason-Gamer et al. 2002), eight North American **StStHH** tetraploids (*E. canadensis* L., *E. elymoides* (Raf.) Swezey, *E. glaucus* Buckley, *E. hystrix* L., *E. lanceolatus, E. trachycaulus* (Link) Gould ex Shinners, *E. virginicus* L., and *E. wawawaiensis* J. Carlson & Barkworth; Mason-Gamer et al. 2002), one Asian **StStYY** tetraploid (*E. ciliaris*; Mason-Gamer at al. 2002); and allohexaploid *E. repens* (Mason-Gamer 2004). New tRNA gene/spacer and *rpo*A sequences were obtained from *E. abolinii* (Drobov) Tzvelev (originating in China; USDA accession PI531555; genome complement **StStYY**), *E. caucasicus* (C. Koch) Tzvelev (Armenia; PI531573; **StStYY**), *E. dentatus* (Hook. f.) Tzvelev (Russia; PI628702; **StStHH**), and *E. mutabilis* (Drobov) Tzvelev (Russia; PI628704; **StStHH**). The chloroplast tRNA genes *trn*T, *trn*L, and *trn*F, along with their intervening noncoding regions, were amplified and sequenced as in Mason-Gamer et al. (2002) using the primers ''a,'' ''b,'' ''c,'' ''d,'' ''e,'' and ''f'' (Taberlet et al. 1991). The *rpo*A gene was amplified and sequenced as in Mason-Gamer et al. (2002) using primers rpoA1, rpoA2, rpoA4, rpoA5, rpoA8, and rpoA9 (Petersen and Seberg 1997). New sequences have been assigned GenBank accession numbers DQ159288–DQ159291 (tRNA genes/spacers) and DQ159332–DQ159335 (*rpo*A).

Comparisons among earlier versions of the three cpDNA data sets (Mason-Gamer et al. 2002) revealed no evidence of conflict among them, consistent with the predominantly clonal, maternal pattern of inheritance of the chloroplast genome in angiosperms (Sears 1980; Hagemann 1992). In the analyses presented here, the data sets were combined and analyzed with PAUP* vers. 4.0b10 (Swofford 2002) using cladistic parsimony with all characters equally weighted. Heuristic searches were run using tree-bisection-reconnection branch swapping, stepwise addition, and simple addition sequence. Gaps in the sequence alignments were treated as missing data. Branch support was estimated using 1000 ''fast bootstrap'' replicates, as implemented in PAUP* vers. 4.0b10 (Swofford 2002). Trees were rooted using *Psathyrostachys*; two earlier cpDNA analyses (Mason-Gamer and Kellogg 1996*a*; Petersen and Seberg 1997) used *Bromus* L. (tribe Bromeae) as an outgroup and both studies provided strong evidence (100% bootstrap in both cases) that *Psathyrostachys* is at the base of the Triticeae cpDNA tree.

The GBSSI data set adds newly acquired data from five tetraploid Eurasian species to existing data from representatives of most of the monogenomic genera of the tribe (Mason-Gamer et al. 1998; Mason-Gamer 2001), from six North American **StStHH** tetraploids (*E. elymoides, E. glaucus, E. lanceolatus, E. trachycaulus, E. virginicus,* and *E. wawawaiensis*; Mason-Gamer 2001) and from hexaploid *E. repens* (Mason-Gamer 2004). New data were obtained from *E. abolinii* (China; PI531555), *E. caninus* L. (Uzbekistan; PI314205), *E. ciliaris* (China; PI531575), *E. dentatus* (Russia; PI628702), and *E. mutabilis* (Russia; PI628704). A 1.3 kilobase portion of the GBSSI gene was amplified, cloned, and sequenced as in Mason-Gamer (2001) using primers F-for, H-for, J-bac, L1-for, L2-bac, and M-bac (Mason-Gamer et al. 1998). Because multiple, distinct nuclear sequences are expected to coexist in allopolyploid individuals, multiple clones per individual (4–12 from each tetraploid, 20+ from *E. repens*) were partially sequenced and compared in an attempt to obtain, and then fully sequence, all variants within each individual. The ten new tetraploid sequences included here have been assigned GenBank accession numbers DQ159322–DQ159331.

The GBSSI data were analyzed using PAUP* vers. 4.0b10 (Swofford 2002). Initial cladistic parsimony analyses were carried out with all characters equally weighted, except that those in ambiguously aligned regions were excluded from the analysis. Nucleotide frequencies, probabilities of different substitution types, and rate variation among sites were estimated on the strict consensus tree from the cladistic analysis using maximum likelihood (ML). Estimated model parameters were used as settings in a subsequent ML search under the general time reversible (GTR) model of sequence evolution (Yang 1994), with an ML-estimated proportion of sites presumed to be invariable (I) and with the remaining sites following a gamma (Γ) distribution (Gu et al. 1995; Waddell and Penny 1996). The initial search settings were: nucleotide frequencies A = 0.240954, C = 0.271580, G = 0.300879, and T = 0.186587; relative character state changes AC = 1.01382, AG = 3.49960, AT = 0.92930, CG = 1.54439, CT = 5.45252, GT = 1.00000; gamma shape parameter describing rate heterogeneity among sites = 0.728590; and proportion of invariable sites = 0.320805. Branch support was estimated based on a bootstrap analysis under a minimum-evolution criterion, with the distances estimated using ML under the same GTR + I + Γ model described above, and using the starting parameters listed above.

RESULTS

Chloroplast DNA Data

The restriction site data set consists of 129 variable sites, and is discussed in detail elsewhere (Mason-Gamer and Kel-

logg 1996*b*). The tRNA gene/spacer PCR products range from 1643 to 1763 base pairs (bp) in length, and the length of the aligned data set (including insertions/deletions [indels]) is 2157 bp. The *rpo*A PCR products range from 1345 to 1371 bp in length, and the length of the aligned data set is 1393 bp. In both cases, sequence alignment was straightforward and all data were included in the analysis.

The **St**-containing allopolyploids are grouped with*Pseudoroegneria,* one of the two hypothesized genome donors (Fig. 1). The *Elymus–Pseudoroegneria* group includes two other genera, *Dasypyrum* (Coss. & Durieu) T. Durand (genome designation **V**) and *Thinopyrum* (genomes **J** and/or **E**), which therefore represent additional potential chloroplast genome donors to *Elymus.* Although bootstrap support for the *Elymus–Pseudoroegneria–Dasypyrum–Thinopyrum* relationship is very weak, the branch lengths indicate that the *Elymus* chloroplast genomes are very similar to those from *Pseudoroegneria.* There is little resolution within the *Elymus–Pseudoroegneria–Dasypyrum–Thinopyrum* clade other than the well-supported *Dasypyrum* and *Thinopyrum* clades themselves, and the branches leading to most of the *Elymus* and *Pseudoroegneria* individuals are very short.

Starch Synthase Data and Tetraploid Elymus

The starch synthase PCR products range from 1193 to 1360 bp in length, and the length of the aligned data set, including indels, is 1598 bp. A total of 114 bp (two regions, 65 and 49 bp) were judged difficult to align such that the positional homology was questionable; these positions were excluded from the analysis.

All of the tetraploid *Elymus* species include a *Pseudoroegneria*-like (**St**) sequence (Fig. 2). The pattern of diversity in the *Pseudoroegneria–Elymus* clade does not fully correspond to either geography or genomic constitution. For example, there are two main subclades within the clade, and each includes both Eurasian and North American *Elymus* tetraploids. Furthermore, some individuals have copies of gene sequences from both of the subclades (*E. lanceolatus* 1a and 1d, and *E. wawawaiensis* 3a and 3b). On the other hand, the three Eurasian **StStHH** species (*E. caninus, E. dentatus,* and *E. mutabilis*) do form a clade within one of the subclades (89% bootstrap support). *Elymus ciliaris* does not fall cleanly into either subclade; preliminary analyses suggest that this is a naturally occurring recombinant sequence, having been recovered from multiple independent PCR reactions.

All of the presumed **StStHH** tetraploids yield *Hordeum*-like (**H**) sequences. The **H**-genome sequences from the North American tetraploids are very similar to one another (0.0036–0.0091 uncorrected pairwise distance) and, along with the sequence from Eurasian *E. dentatus,* form a well-supported clade with *H. californicum* Covas & Stebbins. The three Eurasian **StStHH** *Hordeum*-like sequences are more divergent from one another (0.0437–0.0538 uncorrected pairwise distance), and do not form a clade.

A third clade of tetraploid *Elymus* sequences apparently represents the **Y**-genome sequences from the **StStYY** tetraploids *E. abolinii* and *E. ciliaris.* There is no strong evidence to indicate which of the monogenomic genera are most closely related to the **Y**-genome clade. When the very poorly supported nodes (<50%; unmarked nodes in Fig. 2) are disregarded, many of the basal nodes on the tree collapse, leaving the **Y**-genome clade in an unresolved position relative to the monogenomic genera.

Starch Synthase Data and Hexaploid Elymus repens

Like the **StStHH** tetraploids, the two *E. repens* individuals include both *Pseudoroegneria*-like (1c and 6g) and *Hordeum*-like sequences (1e and 6dd) (Fig. 2). The positions of the *E. repens* **St**- and **H**-genome sequences within their respective clades are not resolved. Sequences 1c and 6g do not form a monophyletic group within the *Pseudoroegneria–Elymus* clade, and 1e and 6dd form only a poorly supported (<50% bootstrap) group within the *Hordeum–Elymus* clade.

Elymus repens sequences appear in two other clades within Triticeae. The third *E. repens* clade groups sequences 6a2 and 1a1 with *Taeniatherum* Nevski with strong support (100% bootstrap). The *Taeniatherum–Elymus* clade is part of a larger clade with *Agropyron* s.s. and *Eremopyrum* (Ledb.) Jaub. & Spach. The fourth *Elymus repens* clade includes sequences 1q and 6hh and does not appear to be closely related to any of the included monogenomic genera. This clade superficially appears to group with *Dasypyrum* and the **Y**-genome clade on the best maximum-likelihood tree, but these relationships lack support (<50% bootstrap; Fig. 2); thus, the donor of the GBSSI sequences in the fourth *E. repens* clade is currently unknown. Earlier analyses of GBSSI exon data within a taxonomically broader sample of grasses (Mason-Gamer 2004) disclosed a fifth *E. repens* sequence type that, while related to sequences in Triticeae, appeared to fall outside of Triticeae (Fig. 3). While this sequence clearly appears to be pooid in origin, the identity of its donor is not revealed by the data currently available.

DISCUSSION

The wheat tribe has been the subject of extensive studies of meiotic behavior in hybrids. Based on these, the monogenomic genera have been delimited according to whether their chromosomes pair at meiosis. The generic definition of the allopolyploid genus *Elymus* is somewhat more complicated. Members of all species of the genus have a *Pseudoroegneria* (**St**) genome, but the other *Elymus* genomes represent diverse sources. Thus, *Elymus* is genomically heterogeneous, as has been further demonstrated in phylogenetic analyses of DNA sequence data.

Chloroplast DNA data are consistent with three possible maternal genome donors: *Pseudoroegneria, Dasypyrum,* or *Thinopyrum* (Fig. 1). As previously suggested (Mason-Gamer et al. 2002), *Pseudoroegneria* is the most likely donor. First, it is consistent with the existing cytogenetic data that formed the basis of the initial hypothesis that the **St** genome is a component of all *Elymus* species. Second, although some studies have suggested that *Thinopyrum* may have been a genome contributor to *E. repens* (e.g., Ørgaard and Anamthawat-Jónsson 2001), molecular data from nuclear genes encoding GBSSI (Fig. 2), phosphoenolpyruvate carboxylase (PepC; Helfgott and Mason-Gamer 2004), and the tissue-ubiquitous form of β-amylase (β-amy; R. J. Mason-Gamer unpubl. data) provide no evidence for the presence of *Thinopyrum*-like or *Dasypyrum*-like sequences in any *Elymus*

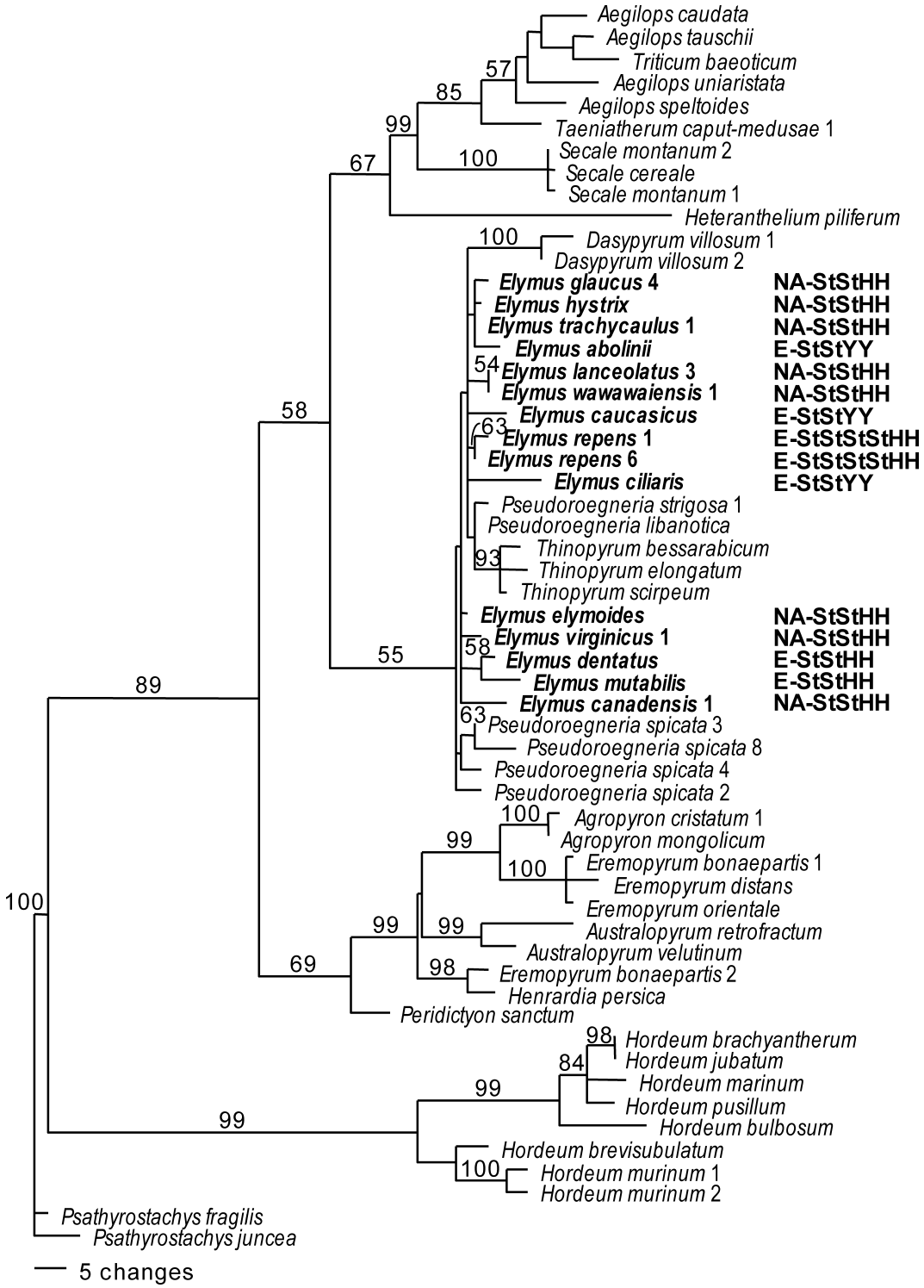

Fig. 1.—Consensus of 63,465 most-parsimonious cpDNA trees based on a combination of data from restriction sites, sequences of the *trn*T, *trn*L, and *trn*F genes and their associated noncoding regions, and sequences of the *rpo*A gene. *Elymus* polyploids are shown in boldface. Labels following the *Elymus* species names indicate whether each species is native to North America (NA) or Eurasia (E), and provide the presumed genomic constitution of each (**StStHH, StStYY,** or **StStStStHH**). Numbers following some species names correspond to specific individual plants within a species, and match the numbers used in previous studies (Mason-Gamer et al. 2002; Mason-Gamer 2004), in which specimen information can be found. Maximum-parsimony bootstrap support values ≥50% are shown above the nodes.

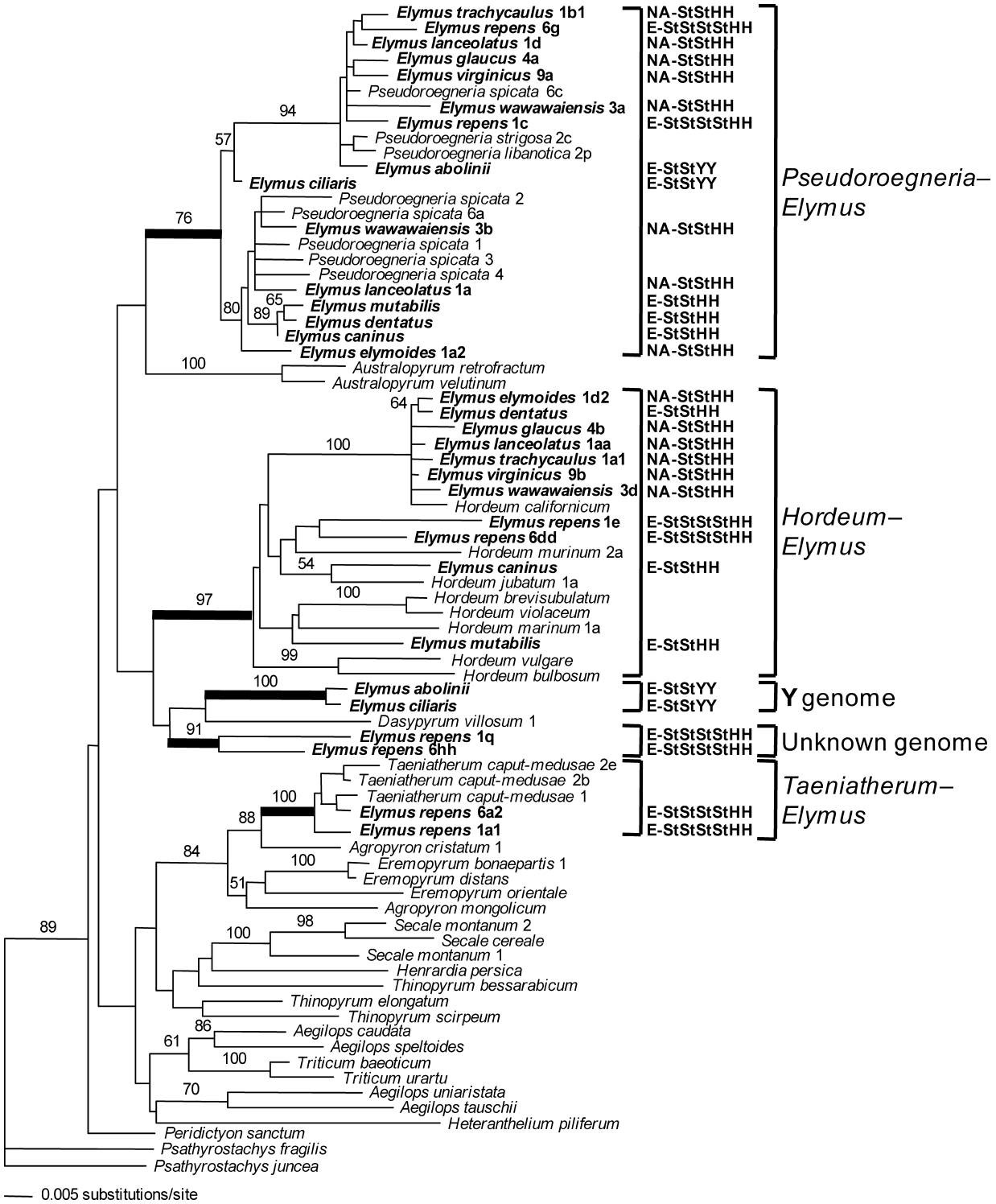

Fig. 2.—Maximum-likelihood tree based on GBSSI sequence data, analyzed under a GTR + I + Γ model of sequence evolution. *Elymus* polyploids are shown in boldface. Numbers following some species names correspond to specific individual plants within a species, and match the numbers used in previous studies (Mason-Gamer 2001, 2004), in which specimen information can be found. The character(s) after each numerical identifier is (are) specific to a single cloned sequence from within that individual. Brackets on the right and the corresponding thick nodes mark all of the clades in which *Elymus* sequences are found; boldface labels between brackets indicate whether each species is native to North America (NA) or Eurasia (E), and provide the presumed genomic constitution of each. Support values ≥50% are shown above the nodes, and were based on a minimum-evolution criterion with distances estimated using maximum likelihood under a GTR + I + Γ model of sequence evolution.

species examined to date. Third, among the available molecular data sets, a *Pseudoroegneria–Dasypyrum–Thinopyrum* group is unique to cpDNA trees, and the level of cpDNA divergence among them is low (Fig. 1). Thus, their cpDNA similarity may reflect introgression of the chloroplast genome from *Pseudoroegneria* into *Thinopyrum* and *Dasypyrum* (Kellogg et al. 1996). The cpDNA data thus highlight the consistent involvement of *Pseudoroegneria* as the maternal genome donor to *Elymus* species of varying polyploid genomic content. This appears to be a general pattern for all **St**-containing allopolyploids examined to date (Redinbaugh et al. 2000; McMillan and Sun 2004).

The nuclear starch synthase gene data show, as expected, multiple genome donors to *Elymus* (Fig. 2). The data support the hypothesized origins of the **StStHH** tetraploids from both North America (*E. elymoides, E. glaucus, E. lanceolatus, E. trachycaulus, E. virginicus,* and *E. wawawaiensis*) and Eurasia (*E. caninus, E. dentatus,* and *E. mutabilis*). Both *Hordeum*-like and *Pseudoroegneria*-like starch synthase gene sequences have been recovered from each presumed **StStHH** tetraploid. The **St**-genome sequences from the North American **StStHH** species are scattered throughout the *Pseudoroegneria–Elymus* clade, and are represented in both of two main subclades that divide the group. The two subclades do not appear to correspond to either geographical or taxonomic boundaries (Mason-Gamer 2001), and some North American individuals contain representatives of both subclades. The **St**-genome sequences from the three Eurasian **StStHH** species, in contrast to the North American species, form a clade. The pattern in the *Hordeum–Elymus* clade is nearly the reverse: the six North American **H**-genome sequences are very similar to one another (and to *H. californicum*), while the **H**-genome sequences from the three Eurasian **StStHH** species are much more diverse. The pattern suggests a single **H**-genome donor to the North American species, while several *Hordeum* species may have been involved in multiple origins of the Eurasian species.

Hybridization and cytogenetic studies have yet to reveal a potential diploid **Y**-genome donor (e.g., Dewey 1984 and references therein). The GBSSI gene sequence data from the two **StStYY** tetraploids, *E. abolinii* and *E. ciliaris,* provide little additional information to help solve the mystery of the **Y** genome's origin. The **Y**-genome clade on the GBSSI tree (Fig. 2) does not show a close relationship to any of the monogenomic genera on the tree, suggesting that none of the included genera are very closely related to the donor of this genome. Preliminary data from genes encoding PepC and β-amy (R. J. Mason-Gamer unpubl. data) also leave the position of the **Y**-genome sequences unresolved.

The GBSSI data from *E. repens* reveal several distinct starch synthase gene copies (Fig. 2). Both of the *E. repens* individuals have a *Pseudoroegneria*-like sequence, consistent with earlier cytogenetic data (Dewey 1970*a*, 1976), and a *Hordeum*-like sequence, consistent with cytogenetic analyses (Cauderon and Saigne 1961) and genomic in-situ hybridization results (Ørgaard and Anamthawat-Jónsson 2001). Furthermore, both of the individuals included here have two additional, phylogenetically distinct, gene copies. One of these forms a well-supported clade with *Taeniatherum* (100% bootstrap). This clade is grouped with *Agropyron* and *Eremopyrum* with moderate (84%) support. Both *Elymus repens* and *Taeniatherum* are widespread introduced weeds in the USA, but they are morphologically very distinct, so the discovery of a *Taeniatherum*-like gene copy in *E. repens* was unexpected. Preliminary analyses of three Eurasian accessions of *E. repens* (R. J. Mason-Gamer unpubl. data) reveal *Taeniatherum*-like sequences in each, suggesting that this gene copy was acquired prior to the introduction of *E. repens* to North America. The analysis also reveals a fourth GBSSI gene copy within *E. repens* (1q and 6hh), which does not group strongly with any other members of the tribe, so its origin remains unknown. While these sequences superficially appear to be related to the *Dasypyrum* and/or **Y**-genome sequences, there is no appreciable support for these relationships (bootstrap <50%).

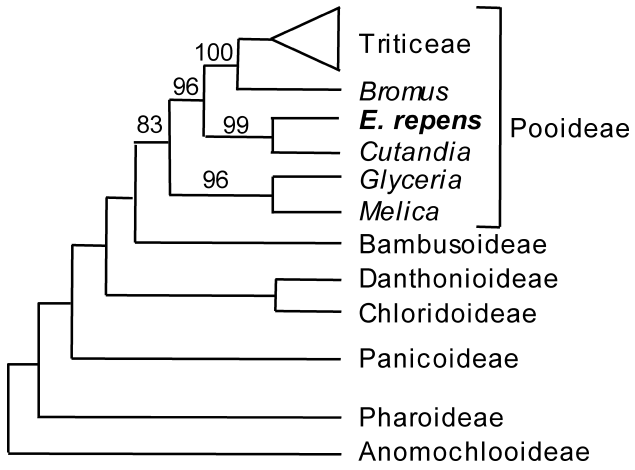

Fig. 3.—Schematic tree showing the placement of an *Elymus repens* sequence variant within Pooideae, but outside of Triticeae. The sequence was found in the individuals numbered 2 and 6 in Fig. 2. The tree topology, redrawn and simplified from Mason-Gamer (2004), was based on exon sequences from the GBSSI gene, analyzed using maximum likelihood under a GTR + I + Γ model of sequence evolution. Bootstrap support values ≥50% are shown for pooid nodes, and were estimated as in Fig. 2.

Previous analyses of *E. repens* GBSSI exon sequences within a broader phylogenetic context (Mason-Gamer 2004) revealed the presence of a fifth, distinct *E. repens* gene copy—one that falls outside of Triticeae (Fig. 3). While the copy is apparently pooid, the sparse sampling within the subfamily does not allow speculation about the identity of its donor. The closest relative within the sample is *Cutandia* Willk., but the related *E. repens* sequence is more divergent from the *Cutandia* sequence than any of the other *E. repens* sequences are from their apparent donors (Mason-Gamer 2004). Thus, a more precise hypothesis regarding the identity of the donor of the fifth *E. repens* sequence awaits a broader sample of GBSSI sequences from within Pooideae, an undertaking being pursued elsewhere (J. I Davis pers. comm.). Another intriguing question about this distinct sequence is whether it was acquired before or after the introduction of *E. repens* to North America over a century ago. With additional *E. repens* samples representing Eurasia, it may be possible to address this interesting issue.

Both of the *E. repens* individuals included here (1 and 6) have all five of the GBSSI sequence variants described above. The variation within this species is thus more com-

plex than expected in an allohexaploid, in which no more than three distinct copies would be expected. Therefore, the origin of the species may have involved other processes, such as introgression, in addition to polyploidy. It remains to be seen whether results based on other genes will be as complex as those from the analyses of the GBSSI data. Data from genes encoding PepC and β-amy will be added to those from GBSSI, and are expected to show whether or not the complex pattern of GBSSI diversity in *E. repens* represents a genome-wide pattern.

Within Triticeae, the **St** genome plays a major role in polyploid evolution—it is a component of many distinct allopolyploid combinations at tetraploid, hexaploid, and octoploid levels. This paper presents a simultaneous analysis of three such genomic combinations, including North American and Eurasian **StStHH** tetraploids, Eurasian **StStYY** tetraploids, and North American representatives of presumed **StStStStHH** *E. repens,* a native of Eurasia. However, these groups represent only a portion of the diversity found among the **St**-containing allopolyploids. A fuller understanding of the role of this genome in the evolution of the tribe will require the use of multiple molecular markers, and the investigation of additional **St**-containing polyploids, including: (1) more representatives within the *Elymus* groups already included here; (2) representatives of additional genome combinations at higher ploidy levels, including the **StStHHYY** and **StStYYPP** hexaploids from Eurasia, the **StStYYWW** hexaploids of Australasia, and the **StStHHNsNsNsNs** octoploid *Pascopyrum smithii*; (3) samples of the putative **St**-containing members of *Thinopyrum*; and (4) **St**-genome autopolyploid representatives of *Pseudoroegneria.* The gradual untangling of the history of polyploidy and hybridization within Triticeae will further enhance our understanding of the tribe's complex and fascinating evolutionary history.

ACKNOWLEDGMENTS

Sincerest thanks to D. Megan Helfgott and Mary Barkworth for ongoing discussions of the Triticeae, to Travis Columbus for reviewing the manuscript, to the USDA for seeds, to the Organizing Committee of the Monocots III/Grasses IV Symposium, and to Pilar Catalán for organizing the Pooideae symposium session. The work was supported by NSF-DEB 9774181.

LITERATURE CITED

CAUDERON, Y., AND B. SAIGNE. 1961. New interspecific and intergeneric hybrids involving *Agropyron. Wheat Inform. Serv.* **12**: 13–14.

CHEN, Q., R. L. CONNER, A. LAROCHE, AND J. B. THOMAS. 1998. Genome analysis of *Thinopyrum intermedium* and *Thinopyrum ponticum* using genomic in situ hybridization. *Genome* **41**: 580–586.

CRONN, R. C., X. ZHAO, A. H. PATERSON, AND J. F. WENDEL. 1996. Polymorphism and concerted evolution in a tandemly repeated gene family: 5S ribosomal DNA in diploid and allopolyploid cottons. *J. Molec. Evol.* **42**: 685–705.

———, R. L. SMALL, T. HASELKORN, AND J. F. WENDEL. 2003. Cryptic repeated genomic recombination during speciation in *Gossypium gossypioides. Evolution* **57**: 2475–2489.

CRONQUIST, A. 1987. A botanical critique of cladism. *Bot. Rev.* **53**: 1–52.

DEWEY, D. R. 1967. Synthetic hybrids of New World and Old World Agropyrons: III. *Agropyron repens* × tetraploid *Agropyron spicatum. Amer. J. Bot.* **54**: 93–98.

———. 1968. Synthetic *Agropyron-Elymus* hybrids: III. *Elymus canadensis* × *Agropyron caninum, A. trachycaulum,* and *A. striatum. Amer. J. Bot.* **55**: 1133–1139.

———. 1970*a*. A cytogenetic study of *Agropyron stipifolium* and its hybrids with *Agropyron repens. Bull. Torrey Bot. Club* **97**: 315–320.

———. 1970*b*. Genome relations among *Elymus canadensis, Elymus triticoides, Elymus dasystachyus,* and *Agropyron smithii. Amer. J. Bot.* **57**: 861–866.

———. 1974. Cytogenetics of *Elymus sibiricus* and its hybrids with *Agropyron tauri, Elymus canadensis,* and *Agropyron caninum. Bot. Gaz.* **135**: 80–87.

———. 1975. The origin of *Agropyron smithii. Amer. J. Bot.* **62**: 524–530.

———. 1976. Derivation of a new forage grass from *Agropyron repens* × *Agropyron spicatum* hybrids. *Crop Sci. (Madison)* **16**: 175–180.

———. 1982. Genomic and phylogenetic relationships among North American perennial Triticeae, pp. 51–81. *In* J. R. Estes [ed.], Grasses and grasslands. Oklahoma University Press, Norman, Oklahoma, USA.

———. 1983*a*. New nomenclatural combinations in the North American perennial Triticeae (Gramineae). *Brittonia* **35**: 30–33.

———. 1983*b*. Historical and current taxonomic perspectives of *Agropyron, Elymus,* and related genera. *Crop. Sci. (Madison)* **23**: 637–642.

———. 1984. The genomic system of classification as a guide to intergeneric hybridization within the perennial Triticeae, pp. 209–279. *In* J. P. Gustafson [ed.], Gene manipulation in plant improvement. Proceedings of the 16th Stadler Genetics Symposium. Plenum Publishing Corporation, New York, USA.

DOYLE, J. J., J. L. DOYLE, A. D. H. BROWN, AND R. G. PALMER. 2002. Genomes, multiple origins, and lineage recombination in the *Glycine tomentella* (Leguminosae) polyploid complex: histone H3-D gene sequences. *Evolution* **56**: 1388–1402.

EMSHWILLER, E., AND J. J. DOYLE. 1998. Origins of domestication and polyploidy in oca (*Oxalis tuberosa*: Oxalidaceae): nrDNA ITS data. *Amer. J. Bot.* **85**: 975–985.

———, AND ———. 2002. Origins of domestication and polyploidy in oca (*Oxalis tuberosa*: Oxalidaceae). 2. Chloroplast-expressed glutamine synthase data. *Amer. J. Bot.* **89**: 1042–1056.

GE, S., T. SANG, B.-R. LU, AND D.-Y. HONG. 1999. Phylogeny of rice genomes with emphasis on origins of allotetraploid species. *Proc. Natl. Acad. Sci. U.S.A.* **96**: 14400–14405.

GU, X., Y.-X. FU, AND W.-H. LI. 1995. Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. *Molec. Biol. Evol.* **12**: 546–557.

HAGEMANN, R. 1992. Plastid genetics of higher plants, pp. 65–96. *In* R. G. Herrmann [ed.], Cell organelles. Springer-Verlag, Vienna, Austria.

HELFGOTT, D. M., AND R. J. MASON-GAMER. 2004. The evolution of North America *Elymus* (Triticeae, Poaceae) allotetraploids: evidence from phosphoenolpyruvate carboxylase gene sequences. *Syst. Bot.* **29**: 850–861.

HULL, D. L. 1979. The limits of cladism. *Syst. Zool.* **28**: 416–440.

JENSEN, K. B. 1990. Cytology and morphology of *Elymus pendulinus, E. pendulinus* ssp. *multiculmis,* and *E. parviglume* (Poaceae: Triticeae). *Bot. Gaz.* **151**: 245–251.

———. 1993. Cytogenetics of *Elymus magellanicus* and its intra- and inter-generic hybrids with *Pseudoroegneria spicata, Hordeum violaceum, E. trachycaulus, E. lanceolatus,* and *E. glaucus* (Poaceae: Triticeae). *Genome* **36**: 72–76.

———. 1996. Genome analysis of Eurasian *Elymus thoroldianus,*

E. melantherus, and *E. kokonoricus* (Triticeae: Poaceae). *Int. J. Pl. Sci.* **157**: 136–141.

KELLOGG, E. A., R. APPELS, AND R. J. MASON-GAMER. 1996. When gene trees tell different stories: the diploid genera of Triticeae (Gramineae). *Syst. Bot.* **21**: 321–347.

LIU, Z.-W., AND R. R.-C. WANG. 1993. Genome analysis of *Elytrigia caespitosa, Lophopyrum nodosum, Pseudoroegneria geniculata* ssp. *scythica,* and *Thinopyrum intermedium* (Triticeae, Gramineae). *Genome* **36**: 102–111.

LÖVE, Á. 1984. Conspectus of the Triticeae. *Feddes Repert.* **95**: 425–521.

LU, B.-R. 1993. Meiotic studies of *Elymus nutans* and *E. jacquemontii* (Poaceae, Triticeae) and their hybrids with *Pseudoroegneria spicata* and seventeen *Elymus* species. *Pl. Syst. Evol.* **186**: 193–212.

———, B. SALOMON, AND R. VON BOTHMER. 1995. Interspecific hybridizations with *Elymus confusus* and *E. dolichatherus,* and their genomic relationships (Poaceae: Triticeae). *Pl. Syst. Evol.* **197**: 1–17.

———, AND R. VON BOTHMER. 1990. Genomic constitution of *Elymus parviglumis* and *E. pseudonutans*: Triticeae (Poaceae). *Hereditas (Lund)* **113**: 109–119.

———, AND ———. 1993. Meiotic analysis of *Elymus caucasicus, E. longearistatus,* and their interspecific hybrids with twenty-three *Elymus* species (Triticeae, Poaceae). *Pl. Syst. Evol.* **185**: 35–53.

MASON-GAMER, R. J. 2001. Origin of North American species of *Elymus* (Poaceae: Triticeae) allotetraploids based on granule-bound starch synthase gene sequences. *Syst. Bot.* **26**: 757–768.

———. 2004. Reticulate evolution, introgression, and intertribal gene capture in an allohexaploid grass. *Syst. Biol.* **53**: 25–37.

———, AND E. A. KELLOGG. 1996*a*. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). *Syst. Biol.* **45**: 524–545.

———, AND ———. 1996*b*. Chloroplast DNA analysis of the monogenomic Triticeae: phylogenetic implications and genome-specific markers, pp. 310–325. *In* P. P. Jauhar [ed.], Methods of genome analysis in plants. CRC Press Inc., Boca Raton, Florida, USA.

———, N. L. ORME, AND C. M. ANDERSON. 2002. Phylogenetic analysis of North American *Elymus* and the monogenomic Triticeae (Poaceae) using three chloroplast DNA data sets. *Genome* **45**: 991–1002.

———, C. F. WEIL, AND E. A. KELLOGG. 1998. Granule-bound starch synthase: structure, function, and phylogenetic utility. *Molec. Biol. Evol.* **15**: 1658–1673.

MCDADE, L. A. 1992. Hybrids and phylogenetic systematics II. The impact of hybrids on cladistic analysis. *Evolution* **46**: 1329–1346.

———. 1998. Hybrids and phylogenetic systematics III. Comparison with distance methods. *Syst. Bot.* **22**: 669–683.

MCMILLAN, E., AND G. SUN. 2004. Genetic relationships of tetraploid *Elymus* species and their genomic donor species inferred from polymerase chain reaction-restriction length polymorphism analysis of chloroplast gene regions. *Theor. Appl. Genet.* **108**: 535–542.

ØRGAARD, M., AND K. ANAMTHAWAT-JÓNSSON. 2001. Genome discrimination by in situ hybridization in Icelandic species of *Elymus* and *Elytrigia* (Poaceae: Triticeae). *Genome* **44**: 275–283.

PETERSEN, G., AND O. SEBERG. 1997. Phylogenetic analysis of the Triticeae (Poaceae) based on *rpo*A sequence data. *Molec. Phylogen. Evol.* **7**: 217–230.

RAUSCHER, J. T., J. J. DOYLE, AND A. D. H. BROWN. 2002. Internal transcribed spacer repeat-specific primers and the analysis of hybridization in the *Glycine tomentella* (Leguminosae) polyploid complex. *Molec. Ecol.* **11**: 2691–2702.

REDINBAUGH, M. G., T. A. JONES, AND Y. ZHANG. 2000. Ubiquity of the **St** chloroplast genome in **St**-containing Triticeae polyploids. *Genome* **43**: 836–852.

SALOMON, B., AND B.-R. LU. 1992. Genomic groups, morphology, and sectional delimitation in Eurasian *Elymus* (Poaceae, Triticeae). *Pl. Syst. Evol.* **180**: 1–13.

———, AND ———. 1994. Genomic relationships between species of the *Elymus semicostatus* group and *Elymus* sensu lato (Poaceae). *Pl. Syst. Evol.* **191**: 199–211.

SANG, T., AND D. ZHANG. 1999. Reconstructing hybrid speciation using sequences of low copy nuclear genes: hybrid origins of five *Paeonia* species based on *Adh* gene phylogenies. *Syst. Bot.* **24**: 148–163.

SEARS, B. B. 1980. Elimination of plastids during spermatogenesis and fertilization in the plant kingdom. *Plasmid* **4**: 233–255.

SEELANAN, T., A. SCHNABEL, AND J. F. WENDEL. 1997. Congruence and consensus in the cotton tribe (Malvaceae). *Syst. Bot.* **22**: 259–290.

SMALL, R. L., AND J. F. WENDEL. 2000. Phylogeny, duplication, and intraspecific variation of *Adh* sequences in New World diploid cottons. *Molec. Phyl. Evol.* **16**: 73–84.

SMEDMARK, J., T. ERIKSSON, R. C. EVANS, AND C. S. CAMPBELL. 2003. Ancient allopolyploid speciation in Geinae (Rosaceae): evidence from nuclear granule-bound starch synthase (GBSSI) gene sequences. *Syst. Biol.* **52**: 374–385.

SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), vers. 4.0b10. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.

TABERLET, P., L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* **17**: 1105–1109.

WADDELL, P. J., AND D. PENNY. 1996. Evolutionary trees of apes and humans from DNA sequences, pp. 53–73. *In* A. J. Locke and C. R. Peters [eds.], Handbook of symbolic evolution. Clarendon Press, Oxford, UK.

YANG, Z. 1994. Estimating the pattern of nucleotide substitution. *J. Molec. Evol.* **39**: 105–111.

ZHANG, X. Y., Y. S. DONG, AND R. R.-C. WANG. 1996. Characterization of genomes and chromosomes in partial amphiploids of the hybrid *Triticum aestivum* × *Thinopyrum ponticum* by in situ hybridization, isozyme analysis, and RAPD. *Genome* **39**: 1062–1071.