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THE ORIGIN AND EVOLUTION OF *ERAGROSTIS TEF* (POACEAE) AND RELATED POLYPLOIDS: EVIDENCE FROM NUCLEAR *waxy* AND PLASTID *rps16*[1]

AMANDA L. INGRAM[2] AND JEFF J. DOYLE

Cornell University, L. H. Bailey Hortorium, 462 Mann Library, Ithaca, New York 14853 USA

Tef (*Eragrostis tef*; Poaceae) is an allotetraploid ($2n = 4x = 40$) cereal crop whose origin within the large genus *Eragrostis* is unknown. Previous studies have suggested a total of 14 wild *Eragrostis* species as potential progenitors. Phylogenetic analysis of sequence data from the nuclear gene *waxy* and the plastid locus *rps16* strongly supports the widely held hypothesis of a close relationship between tef and *E. pilosa*, a wild allotetraploid. *Eragrostis heteromera*, another previously proposed progenitor, is shown by the *waxy* data to be a close relative of one of the tef genomes. Other putative progenitors included in the taxon sample are not supported as closely related to tef. Plastid sequences from five varieties of tef and four *E. pilosa* accessions are identical and therefore are uninformative with respect to the question of multiple origins of these polyploids. The *waxy* phylogeny also resolves the relationships among other allopolyploids, supporting a close relationship between the morphologically similar allotetraploids *E. macilenta*, *E. minor*, and *E. mexicana*. *Eragrostis cilianensis*, another morphologically similar allopolyploid, appears to have shared one diploid progenitor with these species but derived its other genome from an unrelated diploid.

Key words: *Eragrostis*; phylogeny; Poaceae; polyploidy; *rps16*; tef; *waxy*.

Tef (*Eragrostis tef* [Zucc.] Trotter) is an allotetraploid ($2n = 4x = 40$) cereal crop grown primarily in Ethiopia. The grain is used to make a variety of food products, including *injera*, a spongy fermented flatbread that serves as the staple food for most Ethiopians. Despite a long history of cultivation and its importance in Ethiopian culture (Ponti, 1978), little is known about the evolutionary origins of tef. Understanding the origins of crop plants is important because it can provide valuable information for plant breeders who are interested in introgressing agronomically desirable traits from wild relatives of the cultivated species.

A number of investigators have speculated on the origins of tef, using morphological, cytological, and/or biochemical characters and have suggested a total of 14 wild *Eragrostis* species as potential progenitors of the crop (Table 1). Jones et al. (1978) examined morphological and cytological aspects of 41 *Eragrostis* species and concluded that *E. pilosa* was most similar to tef but that *E. aethiopica* also bore striking similarities to the cultigen. Costanza, deWet, and Harlan (1979) examined the relationships among 36 accessions of tef, two *E. pilosa* accessions, and *E. aethiopica* using morphometric methods, and they concluded that *E. pilosa* was far more similar to tef than was *E. aethiopica*. Bekele and Lester (1981) analyzed chromatographic data from leaf phenolic compounds and electrophoretic data of seed proteins from 14 *Eragrostis* species with phenetic methods and suggested that *E. pilosa* was the closest relative of tef. These authors also thought that *E. aethiopica* and *E. barrelieri* were potentially closely related to tef. Tavassoli (1986) conducted cytological examinations of 37 *Eragrostis* species and suggested that *E. aethiopica*, *E. barrelieri*, *E. cilianensis*, *E. mexicana*, *E. minor*, and *E. pilosa* are close relatives of tef based on karyotype morphology.

The general consensus among these studies is that *E. pilosa* is the most likely candidate for the direct wild progenitor of *E. tef*. *Eragrostis pilosa* is a weedy species that occurs throughout the world in tropical and temperate regions and is common in Ethiopia. Cytological investigations have shown that *E. pilosa* is also an allotetraploid and has a karyotype similar to *E. tef* (Tavassoli, 1986). The two species are similar morphologically, and the only documented and consistent morphological distinction between *E. pilosa* and *E. tef* is spikelet shattering. The multi-floreted spikelets of *E. pilosa* readily break apart at maturity as a natural mechanism of seed dispersal, whereas the lemmas, paleas, and caryopses of *E. tef* remain attached to the rachis at maturity and thereby facilitate harvesting (Phillips, 1995). Because of its importance in allowing farmers to control seed dispersal, the transition from shattering to non-shattering is one of the most common traits altered during the domestication process (Heiser, 1973). There is also anthropological evidence that *E. pilosa* is harvested and used as a food source in much the same fashion as tef during times of food scarcity in Ethiopia (Bekele et al., 1995; National Research Council, 1996). Additionally, *E. pilosa* and *E. tef* have been shown to share many identical AFLP markers (Ayele et al., 1999), and it is possible to make interspecific crosses with fully fertile progeny between these species (H. Tefera, Debre Zeit Agriculture Research Center, personal communication). Based on this information, it is likely that tef is a domesticate of *E. pilosa* in which several key agronomic features (e.g., seed mass and spikelet shattering) have been altered through generations of human selection. However, this hypothesis needs further critical testing.

Eragrostis is the largest genus in the Chloridoideae, a well-supported monophyletic subfamily of the Poaceae (GPWG,

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[2] Author for reprint requests (e-mail: ali1@cornell.edu).

TABLE 1. Species identified by previous authors as potential progenitors of *Eragrostis tef*. For authors who examined multiple species, the species identified in the paper as the most likely candidates for the progenitor of tef are marked with an asterisk (*).

Trotter (1938)	Chevalier (1940)	Portères (1958)	Jones et al. (1978)	Costanza, deWet, and Harlan (1979)	Bekele and Lester (1981)	Tavassoli (1986)
E. aethiopica	*E. macilenta*	*E. longifolia*	*E. aethiopica**	*E. aethiopica*	*E. aethiopica**	*E. aethiopica**
			E. barrelieri	*E. pilosa**	*E. barrelieri**	*E. barrelieri**
			E. bicolor		*E. bicolor*	*E. cilianensis**
			E. cilianensis		*E. cilianensis*	*E. mexicana**
			E. heteromera		*E. curvula*	*E. minor**
			E. mexicana		*E. diploachnoides*	*E. pilosa**
			E. minor		*E. heteromera*	
			E. papposa		*E. mexicana*	
			*E. pilosa**		*E. minor*	
					E. papposa	
					*E. pilosa**	
					E. viscosa	

2001). Phylogenetic relationships within *Eragrostis* are poorly understood, and the monophyly of the genus has been called into question in recent phylogenetic analyses of the subfamily (Van den Borre and Watson, 1997; Hilu and Alice, 2001). This large cosmopolitan genus is composed of approximately 350 species (Clayton and Renvoize, 1986), with the greatest species diversity occurring in dry tropical regions. *Eragrostis* species are generally characterized by C_4 photosynthesis, a three-nerved lemma, and paniculate inflorescences. Polyploidy is common in the genus with approximately 69% of species being polyploid (Hunziker and Stebbins, 1986). In addition, chromosome counts at a variety of ploidy levels have been found within a number of morphological species (e.g., Spies, 1982; Bir and Sahni, 1985). *Eragrostis tef* is the only species in the genus cultivated for its grain, but *E. curvula* and tef are grown in many parts of the world as forage grasses or to control soil erosion. Previous attempts at classification within this genus have focused largely on characters such as the manner of spikelet disarticulation or C_4 photosynthesis type (reviewed in Van den Borre and Watson, 1994). However, a cladistic analysis of morphological and anatomical characters has suggested that the mode of spikelet disarticulation has little to do with natural relationships in the group (Van den Borre and Watson, 1994). Actual relationships among the taxa in this analysis may have been obscured by the presence of several allopolyploid taxa (hybrid taxa complicate cladistic analyses due to their often conflicting combinations of morphological character states; McDade, 1990, 1992).

Molecular data can provide useful information regarding the origin of hybrid taxa. Nuclear genes are particularly helpful because they can provide evidence for the affinities of homoeologous genomes in allopolyploid taxa with their diploid progenitors (Doyle and Doyle, 1999). Low-copy nuclear genes have been underexploited relative to plastid or rDNA sequences in studies of plant relationships in the past. However, they have recently emerged as an excellent source of phylogenetically useful characters in polyploids because they often have variable intron sequences that can be used to dissect the relationships among closely related taxa (Small et al., 1998) and seem to exhibit little intergenomic concerted evolution (Cronn, Small, and Wendel, 1999). The phylogenetic utility of the nuclear gene encoding granule-bound starch synthase I (GBSSI; *waxy*) has been demonstrated at a wide range of taxonomic levels in the Poaceae (Mason-Gamer, Weil, and Kellogg, 1998). The exons are variable and alignable at the family level, and the introns provide numerous characters for lower level studies. This gene has also been shown to be single copy in all diploid grasses studied, which alleviates the complications associated with differentiating between paralogous and orthologous copies of the gene. These attributes suggest that this locus could be useful in elucidating relationships among tef and its potential progenitors.

Plastid loci have been an important source of characters for phylogenetic analysis for many years due to the ease of amplification and sequencing (Olmstead and Palmer, 1994). Plastids have been shown to be uniparentally transmitted in most angiosperms (Corriveau and Coleman, 1988), with maternal inheritance in grasses (Mogensen, 1988). Uniparental inheritance limits the plastid genome's utility for identifying the diploid progenitors of allopolyploid taxa, but it makes these sequences valuable for determining which diploid was the maternal progenitor of the polyploid. Plastid data can also be useful in identifying multiple origins of the polyploid in cases in which both diploids have served as the maternal progenitor (e.g., Doyle, Doyle, and Brown, 1990; Soltis et al., 1995). Chloroplast genomes generally evolve slowly (Olmstead and Palmer, 1994), but primer sets have been developed for several loci that are more variable and can be used at the species level. One such locus is *rps16*, which was originally developed by Oxelman, Liden, and Berglund (1997) for use at low taxonomic levels in the Caryophyllaceae.

In this study we have used DNA sequence data from the nuclear gene *waxy* and the plastid locus *rps16* to test previous hypotheses of the origins of *Eragrostis tef*. The lack of consensus on infrageneric relationships in *Eragrostis* complicates the search for progenitors of tef. For this reason, our strategy has been to include as many previously proposed progenitors as possible, supplemented with a broad sample of other *Eragrostis* species that represent the morphological diversity found in the genus. We demonstrate that *waxy* is useful for identifying homoeologous loci in allopolyploid *Eragrostis* species and that *rps16* is able to distinguish between genome groups to identify the maternal progenitor of several *Eragrostis* allopolyploids. These data also support previous hypotheses about the close relationship between *E. tef* and *E. pilosa*.

MATERIALS AND METHODS

Plant materials were obtained from the USDA's National Plant Germplasm System and from personal collections. Data on the species' accession or collection number, voucher number, and provenance have been archived at the Botanical Society of America website in Appendix 1 (http://ajbsupp.botany.

TABLE 2. Morphological characteristics of tef accessions sampled for this study.

Cultivar	Panicle form	Caryopsis color
Addissie	compact	yellow-white
Alba	loose	yellow-white
Karadebi	loose	brown
Variegata	loose	variegated (red/brown)
DZ-01-354	loose	white

org/v90/). A total of 35 *Eragrostis* species were included in the analysis, including several species previously proposed to be close relatives of tef and a broad sample of other species in the genus. Efforts were made to obtain all 14 of the previously proposed progenitors of tef, but only ten were available from personal collections or seed banks. The remaining species were chosen to represent a broad range of the morphological variation observed in the genus, including species exhibiting the major spikelet disarticulation types used to identify subgeneric groupings in previous classifications of *Eragrostis* (e.g., Clayton and Renvoize, 1986). Representatives of both subgenera described in Van den Borre and Watson (1994) are also included. Tef is a morphologically variable species, so accessions from land races that represent a wide cross section of this variation were sampled (Table 2). An improved variety of tef, DZ-01-354, was also included in the taxon sample. Species of *Leptochloa*, *Sporobolus*, and *Uniola* were used as outgroups. Relationships among chloridoid grasses are poorly known, but these genera are all potentially close relatives of *Eragrostis* (van den Borre and Watson, 1997; Hilu and Alice, 2001). Seeds from USDA accessions and the Ethiopian collections were grown in the greenhouse, and the identities of the plants were confirmed from mature plants. Some USDA accessions were misidentified, and corrections are noted in Appendix 1 (http://ajbsupp.botany.org/v90/). Voucher specimens were deposited at Cornell University (BH).

DNA was isolated from 100 mg fresh tissue from greenhouse-grown or wild plants using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) following the manufacturer's instructions. Polymerase chain reaction (PCR) primers wxF and wxM (Mason-Gamer, Weil, and Kellogg, 1998) were used with Ready-to-go PCR beads (Amersham Biosciences, Piscataway, New Jersey, USA) with an annealing temperature of 60°C and an extension time of 1 min 30 s for amplification of four complete and two partial exons and five introns of the *waxy* locus. The PCR products were between 1250 and 1350 base pairs (bp) in length and were cloned with the TOPO TA cloning kit (Invitrogen, Carlsbad, California, USA) after purification on a low-melt agarose gel. Clones were screened by PCR, positive clones were grown overnight in liquid medium, and plasmid preparations were done with the QiaPrep Spin Miniprep Kit (Qiagen). Plasmid DNA was sequenced with the amplification primers and wxS, a new *Eragrostis*-specific sequencing primer (5′-CTG GAG GAG CAG AAG GGC CC-3′) located in exon 10 of the *Zea mays waxy* reference sequence. Plastid *rps16* sequences were obtained by amplifying the region with *rps16*F and *rps16*R2 of Oxelman, Liden, and Berglund (1997) with an annealing temperature of 55°C and an extension time of 1 min in the PCR buffer designed by Pääbo (1990) as modified by Lewis and Doyle (2001). The PCR products were gel-purified with the Qiaquick Gel Extraction Kit (Qiagen) and sequenced directly using the amplification primers. Automated sequencing was performed by the Cornell BioResource Center on an ABI 3700 Automatic Sequencer using Big Dye terminators (Perkin-Elmer Biosystems, Boston, Massachusetts, USA).

Sequences were edited in Sequencher (Gene Codes Corporation, Ann Arbor, Michigan, USA), aligned with DiAlign (Morgenstern, Dress, and Werner, 1996), and adjusted manually. Sequences were deposited in GenBank (accession numbers AY136828–AY136942). Due to the extensive length variation observed in intron regions in *waxy*, sensitivity tests were conducted using a range of gap/change penalties in CLUSTALX (Thompson et al., 1997) to determine the effect of the alignment on the resulting tree topology. Alignment of *rps16* was straightforward. Uncorrected pairwise distances were calculated with PAUP* version 4.0b8 (Swofford, 1998) on the aligned data sets. Unequivocal gaps in each data set were coded as presence/absence characters following the simple gap coding method of Simmons and Ochoterena (2000). Aligned sequences were read into WinClada ver. 0.9.99m24 (Nixon, 1999a) and analyzed with Nona (Goloboff, 1993). Traditional heuristic search strategies (mult*1000; hold/10) and ratchet searches (Nixon, 1999b) were used to find most parsimonious trees. The data sets were analyzed separately because of the large number of allopolyploid taxa in the study. Without extensive genetic mapping, it would be impossible to assign the chloroplast sequences to particular *waxy* homoeologues from the allopolyploids. Bootstrap values were obtained with 1000 replicates of mult 10, hold/1, saving the strict consensus tree for each replicate to estimate branch support. Trees were manipulated in WinClada. Final alignments were deposited in TreeBASE (accession numbers SN1199-3362 and SN1199-3358).

RESULTS

waxy—The PCR amplifications of the *waxy* locus in most allotetraploids included in this study yielded two amplification products of different sizes (approximately 1290 bp and 1320 bp). Multiple clones were sequenced within each size class and were found to be identical or nearly identical in sequence (uncorrected pairwise distance = 0.001–0.006), whereas clones from the different size classes were less similar in sequence (uncorrected pairwise distance = 0.082–0.090). This provided a simple means for identifying putatively homoeologous *waxy* loci in polyploids.

Occasional clones from allopolyploids were sequenced that were obvious results of PCR recombination (Saiki et al., 1988; Bradley and Hillis, 1997), a phenomenon that has been shown to be common when sequencing nuclear genes from allopolyploids (Cronn et al., 2002). When a length difference was present between putative homoeologues, aberrant sequences were generally of intermediate length, and when the sequences were compared to sequences from other clones, they had clearly identifiable points of recombination. In cases where these products were observed, *waxy* was amplified, cloned, and sequenced again to confirm that the PCR recombinant was not reproducible and that apparently non-recombinant sequences could be obtained again.

Interspecific comparisons of the *waxy* sequences showed that orthologous sequences were highly variable even at low taxonomic levels. Uncorrected pairwise divergence values ranged from 0.001 to 0.163, with a mean distance of 0.097. The frequent occurrence of indels in the introns led to complications in aligning these regions, but sensitivity tests showed that variation in gap/change penalties in the alignment had no effect on the resulting topology (data not shown), so all characters were included in the final analysis. The *waxy* sequences yielded a total of 772 informative characters, 46 of which are gap characters. The data set was analyzed with and without the gap characters, and while the gap characters did not affect the ultimate topology of the trees, they did increase the bootstrap values (data not shown).

The cladistic analyses resulted in 451 equally most parsimonious trees of length (L) 2702 steps, consistency index (CI) 0.54, and retention index (RI) 0.79. The strict consensus of these trees (Fig. 1) was well resolved. Alleles at two putatively homoeologous loci from tef and *E. pilosa* were resolved in two separate clades (A and B in Fig. 1), supporting a close relationship between these taxa. *Eragrostis heteromera*, a tetraploid previously proposed to be a progenitor of tef, is shown to be closely related to one of the crop's genomes (B). Other previously proposed progenitors of tef are not shown to be closely related. Homoeologous loci from *E. mexicana*, *E. ma-*

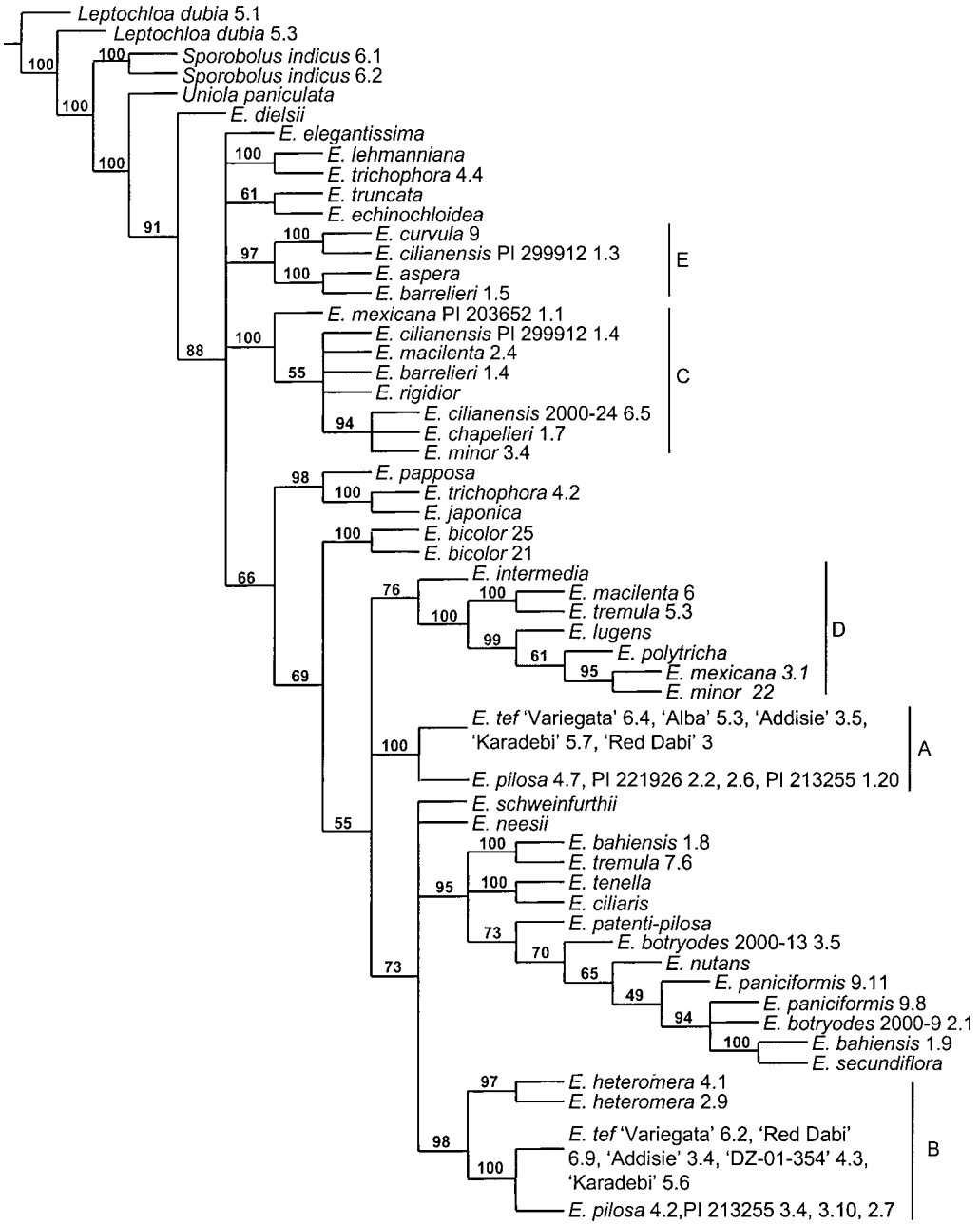

Fig. 1. Strict consensus of 451 most parsimonious trees from a phylogenetic analysis of the *waxy* data set. Numbers above the branches are bootstrap values. All orthologous sequences from *Eragrostis tef* and *E. pilosa* accessions are collapsed to a single branch.

cilenta, and *E. minor*, all of which are polyploids, resolved in two other clades (C and D in Fig. 1). *Eragrostis cilianensis* shared one homoeologue with these polyploids (C), but this tetraploid's other homoeologue was not closely related (E).

rps16—The chloroplast locus *rps16* was much less variable than the *waxy* sequences. Uncorrected pairwise divergence values ranged from 0.000 to 0.034, with a mean distance of 0.013. The alignment of these sequences was straightforward, and five gap characters were included in the data set, producing a total of 64 informative characters. As with the *waxy* data set, the gap characters did not affect the topology of the consensus tree when the results from the complete data set were compared to the results from a phylogenetic analysis excluding the gap characters (data not shown). The cladistic analyses yielded 40 equally most parsimonious trees (L = 93 steps; CI = 0.78; RI = 0.89). Major clades in the strict consensus of these trees (Fig. 2) are labeled to correspond to clades identified in the *waxy* analysis. Because of the large polytomy within *Eragrostis*, it is difficult to assign these groups to particular clades with certainty, so they have been given an ambiguous label (e.g., ''C or E''). The strict consensus is not well resolved and most clades are weakly supported, but all of the tef and *E. pilosa* accessions sequenced for this locus are resolved together (''A or B'' in Fig. 2). These accessions all have identical *rps16* sequences, so there is no evidence that

more than one diploid species contributed a plastid genome to these allopolyploids. This locus is also able to identify the affinity of the maternal progenitor of some allopolyploids. The maternal progenitor of the closely related *E. macilenta*, *E. mexicana*, and *E. minor* seems to be related to an *E. lugens*-type diploid rather than an *E. rigidior*-type diploid (''C or D'' in Fig. 2). These taxa also seem to have a different maternal progenitor than *E. cilianensis* (''C or E'' in Fig. 2), a polyploid sharing one genome with the *E. macilenta*-*E. minor*-*E. mexicana* group. The lack of resolution at lower taxonomic levels within this group, however, makes it impossible to determine which of the genomes identified in the *waxy* analysis represents the maternal progenitor of *E. pilosa* and *E. tef*.

DISCUSSION

This study has shown that the nuclear gene *waxy* is a useful source of characters for resolving phylogenetic relationships in *Eragrostis*. Length differences in homoeologous loci provide an easy means of identifying both copies of the gene in allotetraploids, eliminating the need for sequencing many clones to obtain both homoeologues. This gene is also variable enough to provide information at these low taxonomic levels, though its extensive length variation complicates primary homology assessment during alignment. This study has also demonstrated that *rps16* is not variable enough to be a good source of characters for phylogenetic reconstruction at this taxonomic level in this genus but that it does provide sufficient characters to distinguish between the plastid genomes of the diploid progenitors of some allopolyploids.

Previous hypotheses of the close relationship between *E. tef* and *E. pilosa* based on morphological, biochemical, and biosystematic data were confirmed by this analysis. Orthologous copies of *waxy* from each homoeologous locus for these two species resolve as sister to each other, and the *rps16* sequences from these taxa are also resolved in a well-supported clade. The plastid data do not suggest a bidirectional origin of these allopolyploids, but more thorough sampling in both wild *E. pilosa* and landraces of *E. tef* will be necessary to confirm this result.

Personal observations of *E. pilosa* and *E. tef* specimens and data reported in Ayele et al. (1999) show that these species are generally not distinct morphologically aside from the lack of spikelet shattering in tef. Tef plants are generally larger, have fewer tillers, and take longer to mature, but the range of variation among *E. pilosa* accessions is extensive and usually overlaps with the variation present within cultivated tef. Ayele et al.'s (1999) phenetic analyses of quantitative and qualitative morphological traits of *E. tef* and *E. pilosa* show accessions from each species scattered throughout the phenograms, suggesting that the two species are not morphologically distinct based on the characters measured. The fact that tef does exhibit such a wide range of the variation present in *E. pilosa* (including characters such as lemma color, panicle architecture, and size variation in the grain and the whole plant) suggests that the crop may have been domesticated multiple times, as has been demonstrated in *Phaseolus vulgaris* L. by virtue of the distribution of phaseolin types and isozyme patterns in wild and cultivated accessions (Gepts et al., 1986). It is also possible that there has been gene flow between *E. tef* and *E. pilosa*. Phylogeographic studies will be necessary to assess these scenarios or to determine whether there has simply been extensive differential selection on an initially diverse gene pool to produce the phenotypic variation observed in tef today.

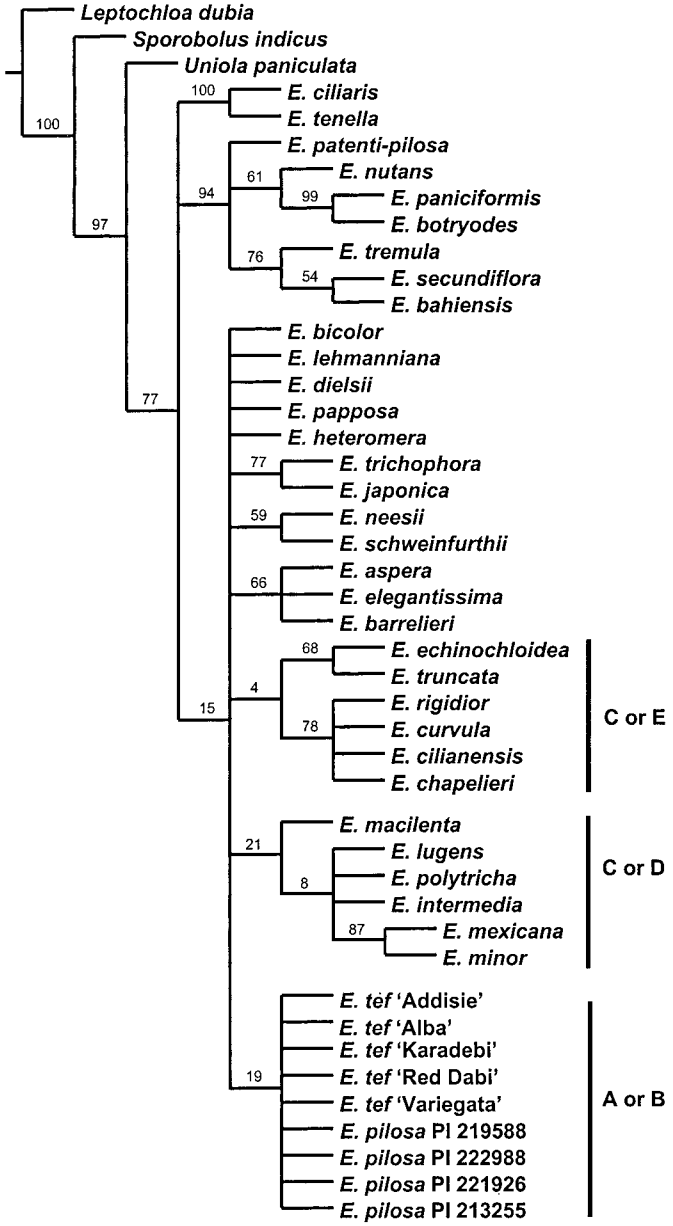

Fig. 2. Strict consensus of 40 most parsimonious trees from the *rps16* data set. Numbers above the nodes are bootstrap values.

An alternative hypothesis for the close relationship between *E. tef* and *E. pilosa* resolved by these data is that *E. pilosa* is a feral derivative of the crop species. While this is impossible to disprove with the current data, this scenario seems unlikely. *Eragrostis tef* is cultivated in a narrow geographic region while *E. pilosa* is distributed throughout tropical and subtropical parts of the world. While tef is currently grown in South Africa and Australia as a forage crop, this is a relatively recent phenomenon. For most of its history as a domesticate, it has been grown almost exclusively in Ethiopia, a country that is isolated from neighboring countries by its high mountains and surrounding deserts. It seems unlikely that *E. pilosa* would

have been able to spread so far and adapt to so many local climates so quickly.

Alleles for *E. heteromera*, an African species that ranges through the eastern part of the continent from Ethiopia southward to South Africa, are resolved as sister to one of the *waxy* homoeologues in tef (B in Fig. 1). Published chromosome counts identify this species as a tetraploid (Tavassoli, 1986). The homoeologous *waxy* sequences from this species are relatively divergent (uncorrected pairwise divergence = 0.042) and include a number of indels scattered throughout the introns, but they clearly are closely related given their position in the *waxy* tree. This species probably is an allopolyploid derived from two closely related diploid species, but more cytological and taxonomic investigations will be necessary to determine the nature of this species' origin. The close relationship of this species with *E. pilosa* and tef shown by this analysis is not surprising: like *E. pilosa*, *E. heteromera* has unequal glumes and spikelets that disarticulate from the bottom with the paleas and lemmas falling together. These characteristics are certainly not unique to these taxa, but they are relatively unusual in the genus. This suite of characters may be useful in the future for identifying diploid taxa that are close relatives of this clade. However, using morphological characters to identify putative diploid progenitors can be problematic due to the complex genetic interactions that often occur in polyploids (Wendel, 2000). It is difficult to predict how combining the genomes of two morphologically distinct diploid species into a single nucleus will affect the phenotype of the resultant polyploid. This complication, in addition to the possibility of extinction and the lack of understanding of phylogenetic relationships within the genus, will make identifying the diploid progenitors of tef difficult. It should be noted, however, that the actual genomes that were involved in creating the polyploid have been identified by sequencing homoeologous *waxy* loci. Even if the diploid progenitors are extinct or are never found, we do have basic knowledge of their genetic composition based on sequence data from the polyploids.

Other previously proposed progenitors of tef that were included in this analysis were not supported as close relatives of the crop from the *waxy* and *rps16* data, but the *waxy* tree provides information on the relationships among a variety of other polyploids. *Eragrostis mexicana*, *E. macilenta*, and *E. minor*, all tetraploids suggested to be close relatives of tef, are shown to be closely related to each other but not to bear any close relationship to the crop species. These species are morphologically similar to each other in that all have glandular foliage and spikelets that disarticulate from the base, leaving behind persistent paleas. *Eragrostis minor* and *E. mexicana* are both weedy species introduced throughout temperate and subtropical regions of the world, but *E. minor* is native to the Old World while *E. mexicana* is a South American species (Phillips, 1995). *Eragrostis macilenta* has a more restricted distribution, ranging across Africa from the Ivory Coast to Ethiopia and southward to South Africa. The morphological similarity to tef that was responsible for these species being suggested as potential progenitors of the crop could be due in part to the relatively close relationship between their D genome diploid progenitor and the B genome progenitor of tef and *E. pilosa*. This *E. mexicana-E. macilenta-E. minor* group may either have been derived from a single allopolyploidization event or from separate polyploidy events involving closely related diploids. The topology of the *rps16* tree suggests that these species all share the same or a closely related maternal progenitor, so the former scenario seems more likely. More detailed comparative studies of the genome structure of these taxa could provide insights into patterns of polyploid evolution in closely related species.

An interesting result from this study is that *E. cilianensis* is not shown to be closely related to *E. minor*, another polyploid with which it is often confused. These species are morphologically similar and are difficult to distinguish in some regions of the world (Phillips, 1995), but it appears that these polyploids have different origins. They may have shared one diploid progenitor (C in Fig. 1), but they derived their other genome from unrelated diploids. The *rps16* tree also shows that they did not share the same maternal progenitor. The shared progenitor is probably responsible for morphological similarities, and it is possible that interspecific hybrids form in some regions, resulting in confusion in species circumscriptions.

More sampling of diploid species will be necessary in future studies to identify the diploid progenitors of the polyploids studied here. Identifying appropriate species is complicated by the prevalence of polyploidy in the genus, a lack of detailed cytological data, and frequent reports of multiple cytological races within a morphological species for those taxa whose cytology has been studied. For instance, different populations of *E. curvula* have been found with chromosome numbers ranging from the diploid level ($2n = 20$) to the octoploid level ($2n = 80$; Spies, 1982). This phenomenon is widespread in the genus. Despite this difficulty, the current study has confirmed the identity of the direct wild progenitor of tef and has eliminated a number of potential progenitors from the pool of candidates, which will be useful in guiding future efforts. *Eragrostis pilosa* may also serve as an important source of comparison for genome-wide studies investigating the effects of domestication and for elucidating the history of cultivated tef. Other interesting groups for future genetic studies have also been identified here, including the *E. mexicana-E. macilenta-E. minor* complex, which may serve as a model for studying the patterns of divergence between closely related polyploids.

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