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Note

Extraordinary Tertiary Constrictions of *Tripsacum dactyloides* Chromosomes: Implications for Karyotype Evolution of Polyploids Driven by Segmental Chromosome Losses

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ABSTRACT

Tripsacum dactyloides ($2n = 2x = 36$) is an ancient tetraploid species. Here we report that *T. dactyloides* chromosomes contain an extraordinary tertiary constriction, which causes a radical and distant separation of a terminal segment from the chromosome. The relationships between extraordinary tertiary constriction and segmental chromosome loss as well as karyotype evolution of polyploid species are discussed.

THE karyotypes of higher eukaryotic species are generally stable. Major karyotypic differences among closely related plant species are often caused by dramatic chromosomal rearrangements. For example, the chromosome number of a species can change due to a chromosomal translocation and loss of a derived chromosome that contains mainly inert genetic material (Darlington 1937). This mechanism was carefully studied using classical cytological methods in the Crepis species (Babcock 1947) and has recently been brilliantly illustrated using a comparative chromosome painting technique in *Arabidopsis thaliana* and its related Brassicaceae species (Lysak *et al.* 2006).

A polyploidization event and its post-diploidization process can result in dramatic karyotype changes because the loss of a chromosomal segment or even a complete chromosome may not be lethal for a newly formed polyploid plant. Chromosome rearrangements, such as intergenomic translocations, were reported in several classical polyploid species, including tobacco and wheat (Kenton *et al.* 1993; Jiang and Gill 1994). Maize (*Zea mays*) provides an excellent example of the complexity of karyotype evolution after a polyploidization event. Maize was derived from an ancient tetraploid (Gaut and Doebley 1997). This tetraploidization event occurred as recently as 4.8 million years ago (Swigonova *et al.* 2004). On the basis of the most recent physical mapping data, the two progenitor species of maize contained $2n = 20$ chromosomes (Wei *et al.* 2007). It is unclear how the 40 chromosomes in the initial tetraploid have decreased to the current 20 chromosomes of maize, despite the extensive comparative mapping effort on maize and its related cereal species (Ahn and Tanksley 1993; Wilson *et al.* 1999; Wei *et al.* 2007).

Tripsacum and Zea are sister genera belonging to the subtribe Maydinae. Tripsacum consists of nine species, including both diploid ($2n = 2x = 36$) and tetraploid ($2n = 4x = 72$) species (Tantravahi 1968). Since the tetraploidization event of maize occurred before the divergence of maize and Tripsacum species (Gaut *et al.* 2000), the diploid Tripsacum species should also represent ancient tetraploids. Thus, karyotypic studies of Tripsacum species may provide clues for the evolutionary history of maize chromosome rearrangements after its tetraploidization event.

Tripsacum dactyloides ($2n = 36$) is cytologically recognized as a diploid species. The 36 *T. dactyloides* chromosomes form 18 bivalents in meiosis without meiotic irregularities (Anderson 1944). During the karyotypic analysis of *T. dactyloides* cv. Pete, we often observed >36 chromosomes in somatic metaphase cells prepared from root tips. Metaphase cells with a clear count of 36 chromosomes were surprisingly rare. Most metaphase cells included one or several very small extra chromosomes (Figure 1a). These small chromosomes appeared to be the satellites associated with the secondary constrictions of nucleolus organizer (NOR) chromosomes, because the satellites can often be distantly separated from the rest of the NOR chromosomes via the secondary constrictions. We performed fluorescence *in situ* hybridization (FISH) using probe pTa71 that contains a 45S ribosomal RNA gene (Gerlach and Bedbrook

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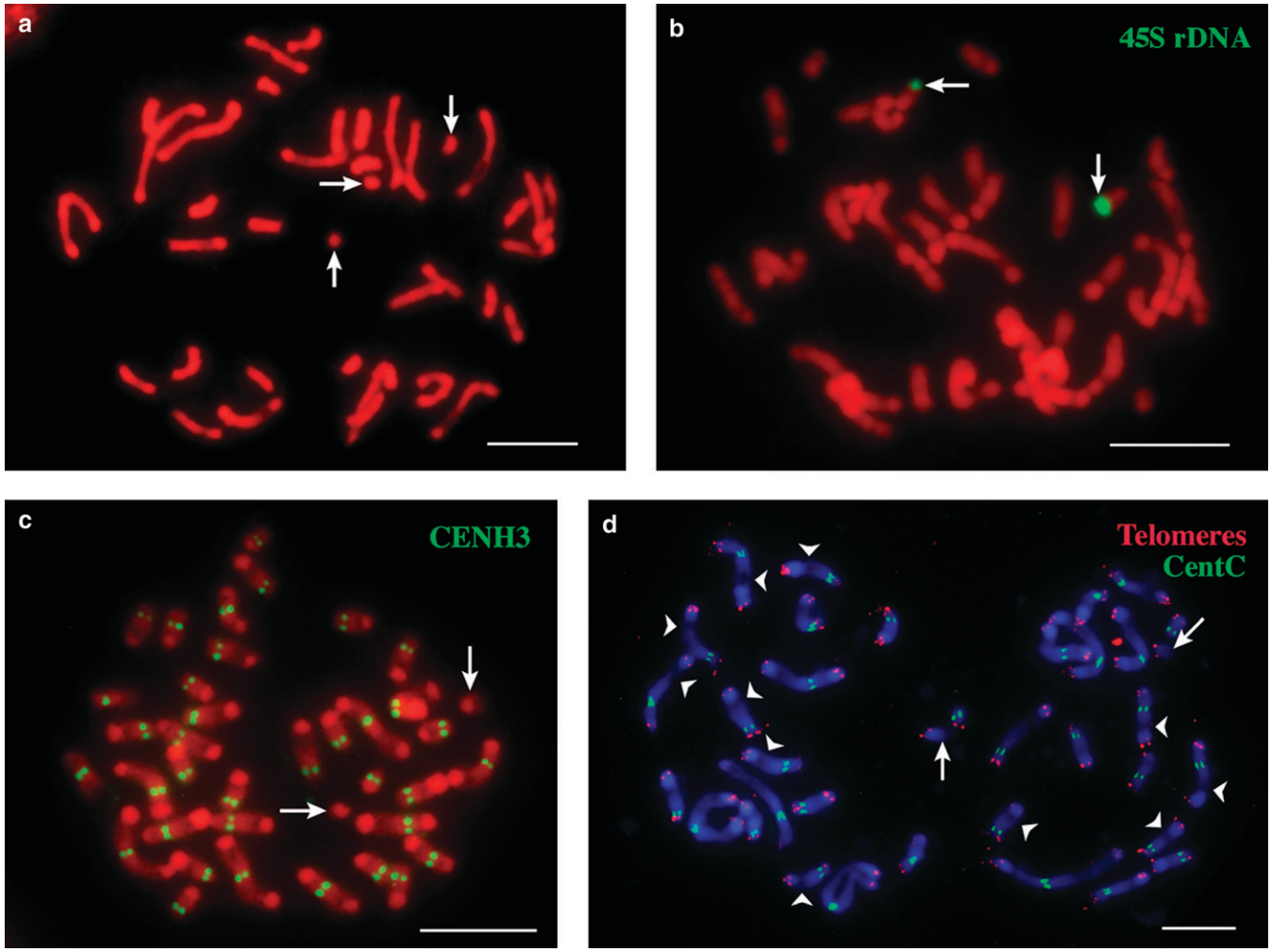

Figure 1.—Tertiary constrictions associated with *T. dactyloides* chromosomes. (a) A somatic metaphase cell of *T. dactyloides*. This metaphase cell contains >36 chromosomes if the 3 small chromosomes (arrows) are counted as independent chromosomes. The chromosomes were stained by 4′,6-diamidino-2-phenylindole (DAPI) and pseudo-colored in red. (b) FISH mapping of 45S rDNA (green signals indicated by arrows). (c) Detection of centromere-specific histone CENH3 by immunofluorescence assay. Two chromosomal segments (arrows) are not independent chromosomes because they lack the CENH3 signals. (d) FISH mapping of telomeric DNA probe pAaT4 and the centromeric probe CentC. Two chromosomal segments (arrows) do not contain CentC signals and contain telomeric signals at only one of the two ends. Tertiary constrictions are associated with most chromosomes and some of the them are indicated by arrowheads. FISH and immunofluorescence assay followed published protocols (Jiang *et al.* 1996; Jin *et al.* 2004). Bars, 10 μm.

1979). The 45S rDNA was mapped to the terminal regions of two small chromosomes that were clearly not associated with any satellites (Figure 1b).

We wondered if the small chromosomes were B chromosomes since B chromosomes were previously reported in several Tripsacum species (Tantravahi 1968). We performed immunofluorescence assay using an antibody against the centromere-specific histone CENH3. This anti-CENH3 antibody was developed in rice (Nagaki *et al.* 2004) and recognizes the CENH3 protein associated with both A and B chromosomes in maize (Jin *et al.* 2005). We did not observe immunofluorescence signals on the small "extra chromosomes" in *T. dactyloides*, suggesting that these chromosomal segments without CENH3 signals are not independent chromosomes (Figure 1c).

The FISH and immunoassay results show that the small chromosomal segments without CENH3 signals were likely separated from chromosomes by tertiary constrictions (TCs). Indeed, TCs were frequently observed on many metaphase chromosomes (Figure 1d) and the distance between the two chromosomal segments separated by a TC was highly variable among different chromosomes and different metaphase cells and in different preparations. Some TCs were extraordinary because the connection between the two chromosome segments were hardly recognized in the metaphase cells (Figure 1d). Such extraordinary TCs have not been previously reported. We also performed FISH analysis using the telomeric DNA probe pAaT4 (Richards and Ausubel 1988) and the CentC repeat that is specific to the centromeres of both maize (Ananiev *et al.* 1998) and

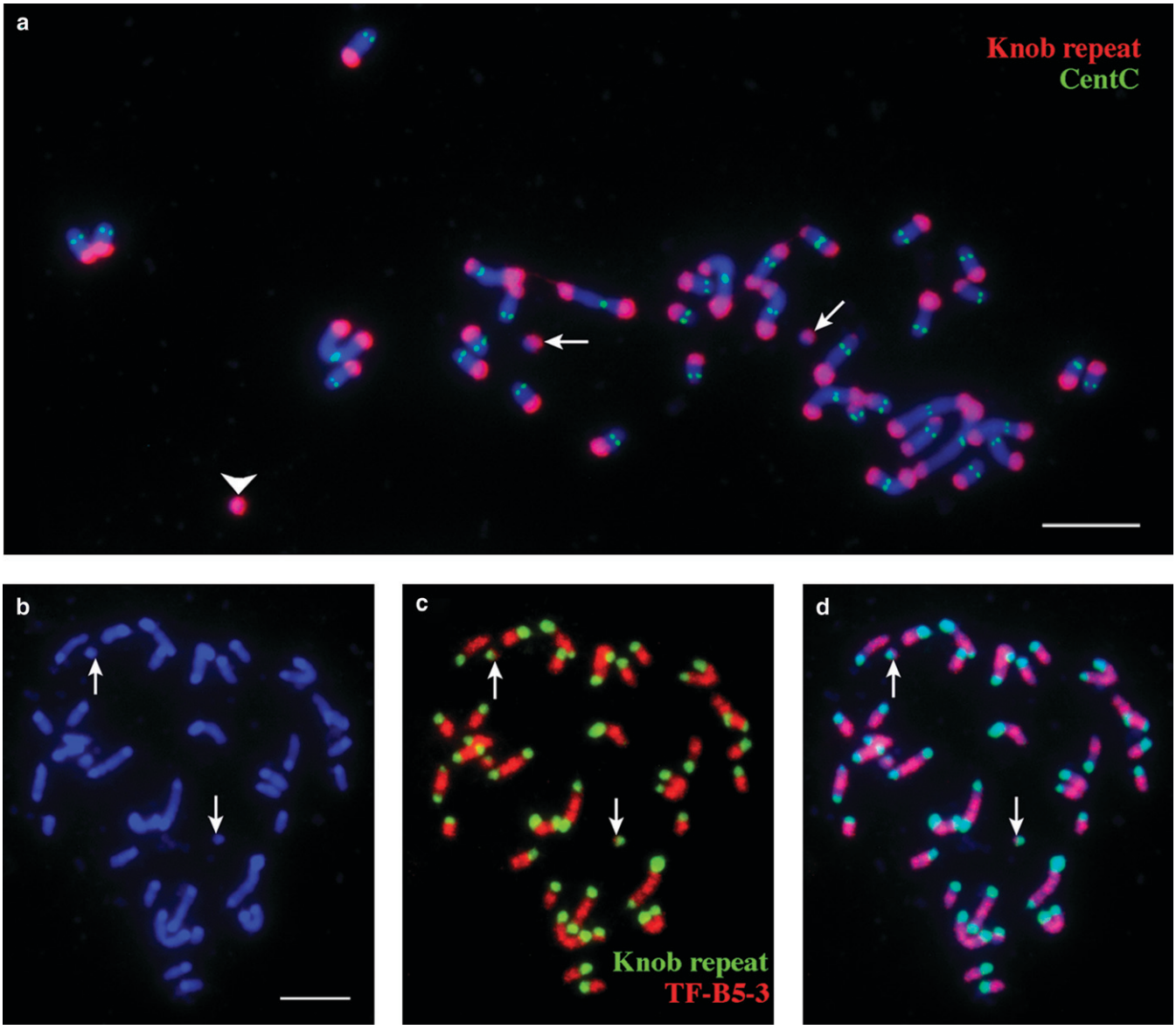

FIGURE 2.—FISH mapping of knob repeat and retroelement TF-B5-3 on the chromosomal segments separated by tertiary constrictions. (a) FISH analysis of probe pTd-8 containing the 180-bp maize knob repeat (pink) and the centromeric repeat CentC (turquoise). A chromosomal segment (arrowhead) is covered almost completely by the knob signals. Two other chromosomal segments (arrows) are not fully covered by the signals from the knob repeat. (b) DAPI staining of a metaphase cell. (c) FISH analysis of knob repeat (green) and retrotransposon probe TF-B5-3 (red). (d) Merged image of b and c. TF-B5-3 signals are visible on two chromosomal segments (arrows) separated by tertiary constrictions. Bars, 10 μm.

T. dactyloides chromosomes (LAMB and BIRCHLER 2006). Some chromosomal segments did not show CentC signals and contained telomeric signals at only one of the two ends (Figure 1d). These results confirmed that these chromosomal segments are not independent chromosomes.

To reveal the DNA composition of the chromosomal segments separated by the TCs, we performed FISH analysis using probe pTd-8 containing the 180-bp maize knob repeat cloned from *T. dactyloides*. A large block of the knob repeat was found either at one end or at both ends of every *T. dactyloides* chromosome (Figure 2a). The chromosomal segments separated by TCs were always associated with a block of the knob repeat. The 18 different *T. dactyloides* chromosomes can be grouped into two types on the basis of the location of the knobs: type I with knobs on both arms and type II with a knob only on one arm (Figure 3). The 45S rDNA is located on a pair of the type II chromosomes.

We noted that some of the chromosomal segments separated by TCs were not completely covered by the FISH signals from the knob repeat (Figure 2a), indicating that the knob repeats account for only part of these chromosomal segments. We performed FISH using probe TF-B5-3 that contains a retrotransposon distributed almost uniformly in the *T. dactyloides* genome (LAMB and BIRCHLER 2006). This probe produced very

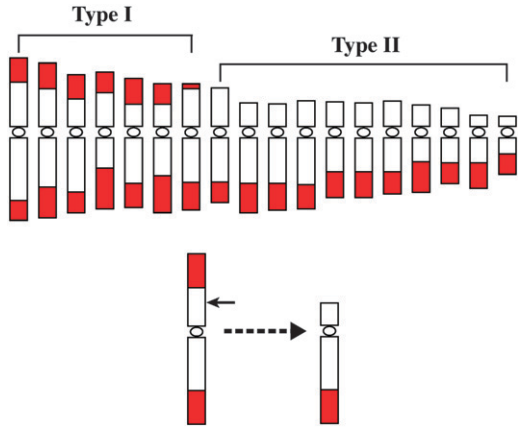

Figure 3.—An ideogram of the karyotype and a model of segmental chromosome loss in *T. dactyloides*. Type II chromosome is the product of a terminal deletion of a type I chromosome. The small arrow points to the deletion breakpoint that is possibly linked to a tertiary constriction.

faint or no signals on the knob regions of *T. dactyloides* chromosomes (Figure 2c). The FISH results confirmed that most observed chromosomal segments separated by TCs contain additional chromatin in addition to the knob repeats (Figure 2, b–d).

Since TCs were never observed on the short arms of type II chromosomes, it appears that the type II chromosomes evolved from the type I chromosomes by loss of a terminal segment (Figure 3). We propose that type II chromosomes are products of terminal deletions of type I chromosomes and that the breakpoints of such terminal deletions are associated with the extraordinary TCs located on the type I chromosomes (Figure 3). This hypothesis is supported by the fact that the sizes of the short arms of the type II chromosomes match well with the sizes of the arms of type I chromosomes without the terminal segments separated by the TCs (Figure 1d and Figure 3). The heterochromatic knobs in *T. dactyloides* and maize contain the same 180-bp repeat (Lamb and Birchler 2006). It is interesting to note that the large knobs in maize are located mainly in interstitial chromosomal regions (Buckler *et al.* 1999). Both terminal and interstitial knobs were observed on chromosomes of *Zea diploperennis* ($2n = 20$) (Lamb *et al.* 2007). Chromosomal mapping of knobs in other Zea and Tripsacum species will reveal the impact of loss of terminal knobs on karyotype evolution of both maize and *T. dactyloides*.

Although the proposed terminal deletions in *T. dactyloides* can result in loss of the telomeric heterochromatin as well as subtelomeric euchromatin, the ancient polyploid nature of the *T. dactyloides* genome may prevent the lethality of such terminal deletions. In addition, all Tripsacum species are perennials with well-developed underground rhizomes that result in asexual propagation (Tantravahi 1968). Asexual propagation would favor retention of chromosomal mutations that may negatively affect transmission of the chromosome in meiosis but have other selective advantages for the plant.

Leitch and Bennett (2004) recently showed that many polyploid species contain less DNA than the combined DNA of the parental species. The loss of DNA, or genome downsizing, following polyploidization may be a widespread phenomenon in nature (Leitch and Bennett 2004). How the genome of a polyploid species becomes downsized is unknown. It may be caused by one or a combination of several documented molecular mechanisms, including elimination of specific DNA sequences (Liu *et al.* 1998; Ozkan *et al.* 2001) or retrotransposon-based illegitimate recombinations (Devos *et al.* 2002). The karyotyping results of *T. dactyloides* show that segmental chromosome loss is a potential mechanism that can result not only in the downsizing of the genome but also in major changes to the karyotype of a polyploid species.

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