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# Comparative analysis reveals chromosome number reductions in the evolution of African bermudagrass (*Cynodon transvaalensis* Burtt-Davy)

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## Abstract

African bermudagrass (*Cynodon transvaalensis* Burtt-Davy) (2n = 2x = 18) belongs to the genus *Cynodon*, tribe Cynodonteae, subfamily Chloridoideae in the grass family Poaceae. The species is frequently crossed with common bermudagrass (*Cynodon dactylon* Pers.) in developing high-quality hybrid turf cultivars. Molecular resources for *C. transvaalensis* are scarce; thus, its genomic evolution is unknown. Recently, a linkage map consisting of 1278 markers provided a powerful tool for African bermudagrass genomic research. The objective of this study was to investigate chromosome number reduction events that resulted in the nine haploid chromosomes in this species. Tag sequences of mapped single nucleotide polymorphism markers in *C. transvaalensis* were compared against genome sequences of *Oropetium thomaeum* (L.f.) Trin. (2n = 2x = 20), a genomic model in the Cynodonteae tribe. The comparative genomic analyses revealed broad collinearity between the genomes of these two species. The analyses further revealed that two major interchromosomal rearrangements of the paleochromosome ρ12 (ρ1–ρ12–ρ1 and ρ6–ρ12–ρ6) resulted in nine chromosomes in the genome of *C. transvaalensis*. The findings provide novel information regarding the formation of the initial diploid species in the *Cynodon* genus.

**Key words:** *Cynodon*, evolution, linkage map, chromosome rearrangement, nested chromosome fusion

## Résumé

Le *Cynodon transvalalensis* Burtt-Davy (« African bermudagrass ») (2n = 2x = 18) appartient au genre *Cynodon*, à la tribu des Cynodonteae, à la sous-famille des Chloridoideae au sein de la famille des Poaceae. Cette espèce est importante car elle est fréquemment croisée avec le chiendent pied-de-poule (*Cynodon dactylon* Pers.) pour développer des cultivars de gazon de grande qualité employés pour les terrains de sports, de golf ou résidentiels. Les ressources moléculaires pour le *C. transvaalensis* sont rares et, en conséquence, son évolution génomique est inconnue. Récemment, une carte de liaison génétique comprenant 1278 marqueurs a fourni un outil puissant pour la recherche génomique chez cette espèce. L'objectif de cette étude était d'étudier les évènements de réduction chromosomique produisant un génome à neuf chromosomes haploïdes chez cette espèce. Les séquences-étiquettes (« tag sequences ») pour les marqueurs SNP (polymorphismes mononucléotidiques) chez le *C. transvaalensis* ont été comparés aux séquences génomiques de l'*Oropetium thomaeum* (L.f.) Trin. (2n = 2x = 20), une espèce modèle au sein de la tribu des Cynodonteae. Les analyses génomiques comparées ont révélé une colinéarité considérable entre les génomes de ces deux espèces. Les analyses ont également montré que deux réarrangements interchromosomiques majeurs du paléo-chromosomes ρ12 (ρ1–ρ12–ρ1 and ρ6–ρ12–ρ6) auraient produit les neuf chromosomes présents dans le génome du *C. transvaalensis*. Ces résultats contribuent des informations nouvelles sur la formation des espèces diploïdes initiales au sein du genre *Cynodon*. [Traduit par la Rédaction]

**Mots-clés :** *Cynodon*, évolution, carte de liaison, réarrangement chromosomique, fusion chromosomique nichée

## Introduction

African bermudagrass (*Cynodon transvaalensis* Burtt-Davy) (2n = 2x = 18) is indigenous to the southwestern Transvaal Orange Free State and the northern part of the central Cape Province of South Africa (Harlan et al. 1970*a*), primarily found near wet areas, such as river banks (Harlan et al. 1970*b*). Compared with other *Cynodon* species, *C. transvaalensis* is unique in its small size, yellow–green color, erect narrow leaves, and two to four racemes per inflorescence (Harlan et al. 1970*a*). Its sod-forming habit and tolerance to low mowing heights

make it suitable for use on golf course putting greens (Gerken 1994). However, its high water and nutrient demand, thatch buildup tendency, and the decline of turfgrass quality in summer limit its use (Taliaferro 1992). In fact, the primary use of African bermudagrass is to serve as a parent crossing with common bermudagrass (*Cynodon dactylon* var. *dactylon* Pers.) to produce interspecific hybrid bermudagrass (*C. dactylon* × *C. transvaalensis*) cultivars possessing excellent turfgrass quality and stress tolerance. A number of interspecific hybrid turf bermudagrass cultivars have been extensively used on golf courses, sports fields, home lawns, and municipal parks (Burton 1991; Taliaferro 1992, 2003).

Grass genomes have evolved from a common ancestor with a base chromosome number of seven, which underwent a series of whole-genome duplications (WGDs), nested chromosome fusions (NCFs), and rearrangements that led to an x = 12 intermediate ancestor (Luo et al. 2009). It is widely accepted that the rice (*Oryza sativa* L.) genome resembles the karyotype of the x = 12 ancestor before separating into the Bambusoideae, Ehrhartoideae, and Pooideae and the Panicoideae, Arundinoideae, Chloridoideae, Centothecoideae, Micrairoideae, Aristidoideae, and Danthonioideae clades (Kim et al. 2009; Luo et al. 2009; F. Wang et al. 2015). It has been proposed that the common ancestor of grasses is a rice-like species with 12 basic paleoancestor chromosomes (ρ) (Salse et al. 2008; Luo et al. 2009; Murat et al. 2010). The Poaceae family includes the major food crops, wheat (*Triticum* spp.), maize (*Zea mays* L.), and rice for most parts of the world, as well as species used for biofuel and turfgrass (Gaut 2002). The subfamily Chloridoideae contains more than 1300 species (Gaut 2002), especially the economically important warm-season turfgrass species such as bermudagrass (*Cynodon* spp.) and zoysiagrass (*Zoysia* spp.). African bermudagrass belongs to the genus *Cynodon*, subtribe Eleusininae, tribe Cynodonteae, subfamily Chloridoideae, and family Poaceae (Harlan et al. 1970*a*; Taliaferro et al. 1997; Soreng et al. 2015). The *Cynodon* species have a basic chromosome number of nine, with African bermudagrass being a diploid (2n = 2x = 18) species and common bermudagrass predominantly a tetraploid (2n = 4x = 36) species (Forbes and Burton 1963). Although lagging behind other subfamilies, the evolutionary history of the Chloridoideae subfamily has drawn attention in molecular investigations recently (Srinivasachary et al. 2007; F. Wang et al. 2015; Huang et al. 2016; Fang et al. 2020). The 12-to-9 chromosome reduction in the *Cynodon* genus has been an intriguing evolutionary question. Comparative mapping studies in zoysiagrass revealed two NCFs (ρ6–ρ9–ρ6 and ρ2–ρ10–ρ2) that explained the reduction of the basic chromosome number from 12 to 10 (F. Wang et al. 2015; Huang et al. 2016). Recently, a comparative genomic analysis among tetraploid *C. dactylon* (2n = 4x = 36), *Oryza sativa* (2n = 2x = 24), and *Oropetium thomaeum* (2n = 2x = 20) shed light on the mechanism of the 10-to-9 chromosome reduction in *Cynodon* with two NCFs (ρ1–ρ12–ρ1 and ρ6–ρ12–ρ6) (Fang et al. 2020). However, no information is available regarding the chromosome number reduction in diploid African bermudagrass (2n = 2x = 18). A high-density African bermudagrass linkage map that consists of 1278 markers (single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs)) has been published recently (Yu et al. 2021). The tag sequences (64 bp in length) of the mapped SNPs provide useful landmarks for comparative genomic studies in revealing the evolutionary history of the African bermudagrass genome. Accordingly, the objective of this study was to investigate chromosome reduction events that resulted in the genome of *C. transvaalensis* through comparison with the fully sequenced genome of *Oropetium thomaeum* (2n = 2x = 20), which is a botanical model species within the Cynodonteae tribe (VanBuren et al. 2015, 2018).

## Materials and methods

The linkage map used in this study was constructed using the "OKC1163" $S_1$ population with 1246 SNP and 32 SSR polymorphic markers (Yu et al. 2021). Genomic synteny between African bermudagrass and *Oropetium thomaeum* was compared to understand the evolutionary history of grasses within the Cynodonteae tribe. The 64 bp sequences of mapped SNP markers in African bermudagrass were compared against genome sequences of *Oropetium thomaeum* (V2.1) (VanBuren et al. 2018) using the Nucleotide Basic Local Alignment Search Tool (BLASTn) (Blast-2.8.1+) with default settings with an *e*-value cutoff at $1 \times 10^{-8}$ (F. Wang et al. 2015; Huang et al. 2016). The *Oropetium thomaeum* reference genome was downloaded from CoGe (https://genomevolution.org/coge/). The top hits of SNP marker sequences showing synteny to the genome of *Oropetium thomaeum* were extracted for comparative analysis. A genomic synteny dot plot was generated using R (version 3.5.2) (R Core Team 2018) with customized scripts based on Fang et al. (2020). A Circos plot was generated in R using the circlize package (Gu et al. 2014).

## Results

Among the 1246 mapped SNPs in the linkage map of *C. transvaalensis*, 163 markers (13.1%) were unambiguously aligned to the *Oropetium thomaeum* genome (Table S1). Genomic synteny between these two species is shown in Fig. 1 and Table 1. Overall, each *C. transvaalensis* linkage group (LG) clearly corresponded in synteny to one or two chromosomes of *Oropetium thomaeum*. Particularly, *C. transvaalensis* LG3 corresponded to the two arms of Ot3 with its central region aligned to one end of Ot10, suggesting that *C. transvaalensis* LG3 may have evolved from the fusion of a segment of the ancestral chromosome corresponding to Ot10 and the ancestral chromosome corresponding to Ot3. *Cynodon transvaalensis* LG6 primarily corresponded to Ot2, but with one end of LG6 showing inverse synteny with the end of Ot10. In addition, sparse yet obvious collinearity was observed between three *C. transvaalensis* LGs and three *Oropetium thomaeum* chromosomes. For example, both *C. transvaalensis* LGs5 and 9 were aligned to one arm of Ot9 and Ot5, respectively, while LG7 was primarily aligned to Ot6. Only two SNPs were aligned between Ot8 and LG8.

Although Ot10 was involved in the abovementioned interchromosomal rearrangements, the number of SNPs on the *C. transvaalensis* linkage map aligned to Ot10 was low (n = 6; Table 1). To further explore the 10-to-9 chromosome

**Fig. 1.** Genomic synteny between *Cynodon transvaalensis* LGs and *Oropetium thomaeum* chromosomes. The *x*-axis shows the genetic location of markers on LGs of *C. transvaalensis* in centimorgans (cM); the *y*-axis indicates the physical position of markers on *Oropetium thomaeum* chromosomes in base pair (bp). Each dot represents an SNP marker.

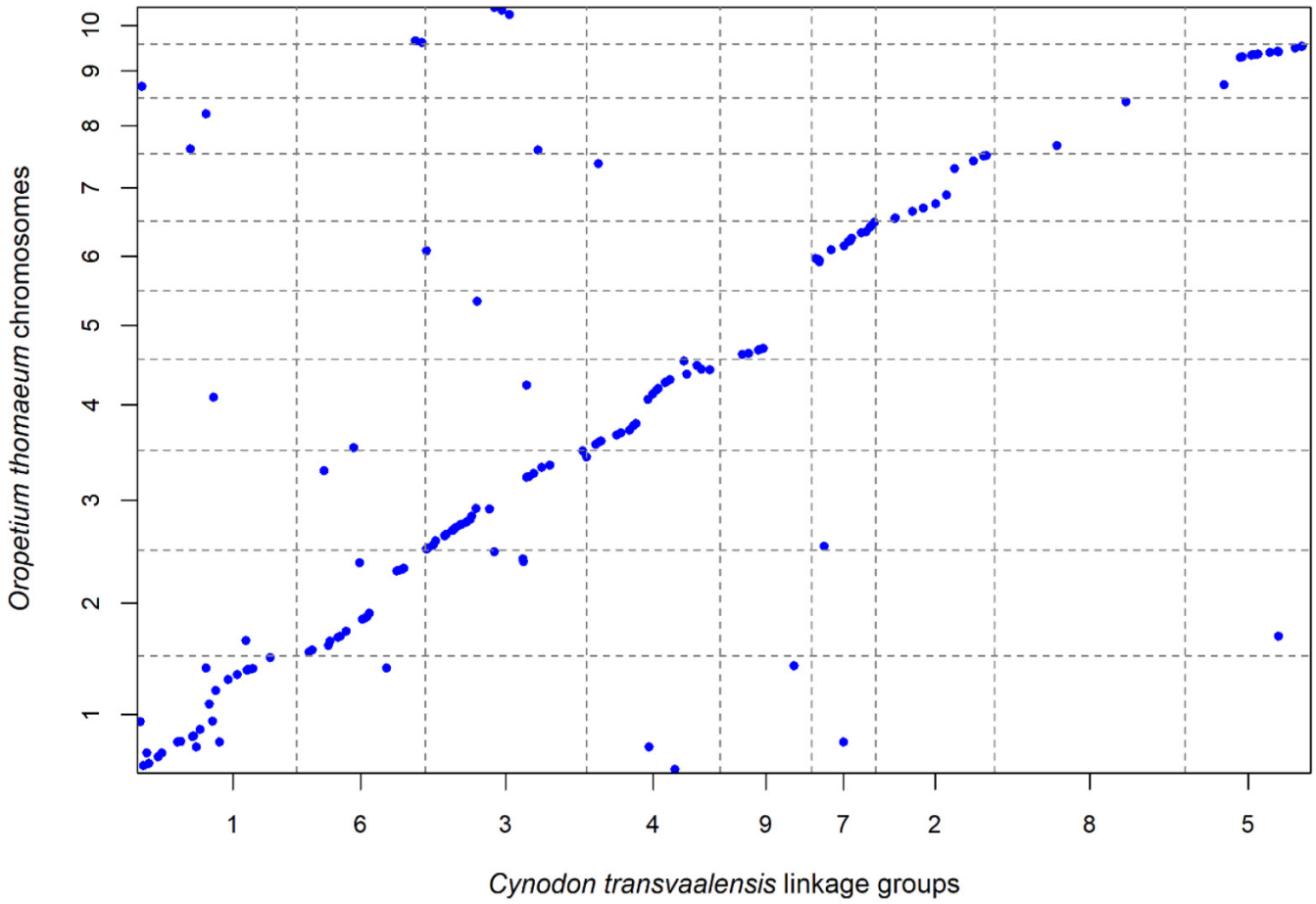


**Table 1.** Genomic comparisons between linkage groups (LGs) of *C. transvaalensis* and corresponding chromosomes of *Oropetium thomaeum* (Ot) and *Oryza sativa* (Os).

| *Cynodon transvaalensis* LG | *Oropetium thomaeum* chromosomes | No. of aligned SNPs | *Oryza sativa* chromosomes |
|---|---|---|---|
| LG 1 | Ot1 | 25 | Os2 (ρ2), Os10 (ρ10) |
| LG 2 | Ot7 | 10 | Os7 (ρ7) |
| LG 3 | Ot3, Ot10 | 28, 3 | Os1 (ρ1), Os12 (ρ12) |
| LG 4 | Ot4 | 21 | Os3 (ρ3) |
| LG 5 | Ot9 | 14 | Os8 (ρ8) |
| LG 6 | Ot2, Ot10 | 16, 3 | Os6 (ρ6), Os9 (ρ9), Os12 (ρ12) |
| LG 7 | Ot6 | 16 | Os4 (ρ4) |
| LG 8 | Ot8 | 2 | Os11 (ρ11) |
| LG 9 | Ot5 | 6 | Os5 (ρ5) |

reduction related to Ot10, we investigated all the raw SNPs generated by Yu et al. (2021) that aligned to Ot10 and reconstructed LGs using JoinMap 5.0 (Van Ooijen 2006). A total of 304 SNPs aligned to Ot10 were recovered for linkage analysis, and 111 of them were successfully grouped into two LGs (Table S2). Among the 111 SNPs, three from one LG were also mapped on LG3, and four from the other LG were also mapped on LG6. Therefore, these two LGs were named LGs 3-1 and 6-1, respectively (Fig. 2; Table S2). Due to the limited number of markers shared between LGs 3 and 3-1, and LGs 6 and 6-1, we combined markers from LGs 3 and 3-1 as well as LGs 6 and 6-1 to construct two new LGs to investigate how the ancestral chromosome corresponding to Ot10 was involved in the interchromosomal rearrangements in these two LGs.

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**Fig. 2.** Distribution of SNP markers between LG 3 and its derivatives (3-1 and 3-2), LG 5 and its derivatives (5-1 and 5-1-1, 5-2, and 5-2-1), LG 6 and its derivatives (6-1), LG 8 and its derivatives (8-1 and 8-2), and LG 9 and its derivatives (9-1 and 9-2) revealed chromosomal rearrangements of *Cynodon transvaalensis*. The LGs 3-1, 5-1, 5-2, 8-1, and 9-1 were constructed based on SNP markers that were successfully aligned to *Oropetium thomaeum* chromosomes 10, 9, 8, and 5 that shared markers with LGs 3, 5, 8, and 9, respectively. LG 3-2 included the markers from LGs 3 and 3-1; LG 5-1-1 included markers from LGs 5 and 5-1; LG 5-2-1 included the markers from LGs 5 and 5-2; LG 8-2 combined all the markers from LGs 8 and 8-1; LG 9-2 combined all the markers from LGs 9 and 9-1. The ruler on the left indicates the genetic distance in centimorgans (cM). Black dots connected with lines in different colors indicate the same markers mapped on related LGs.

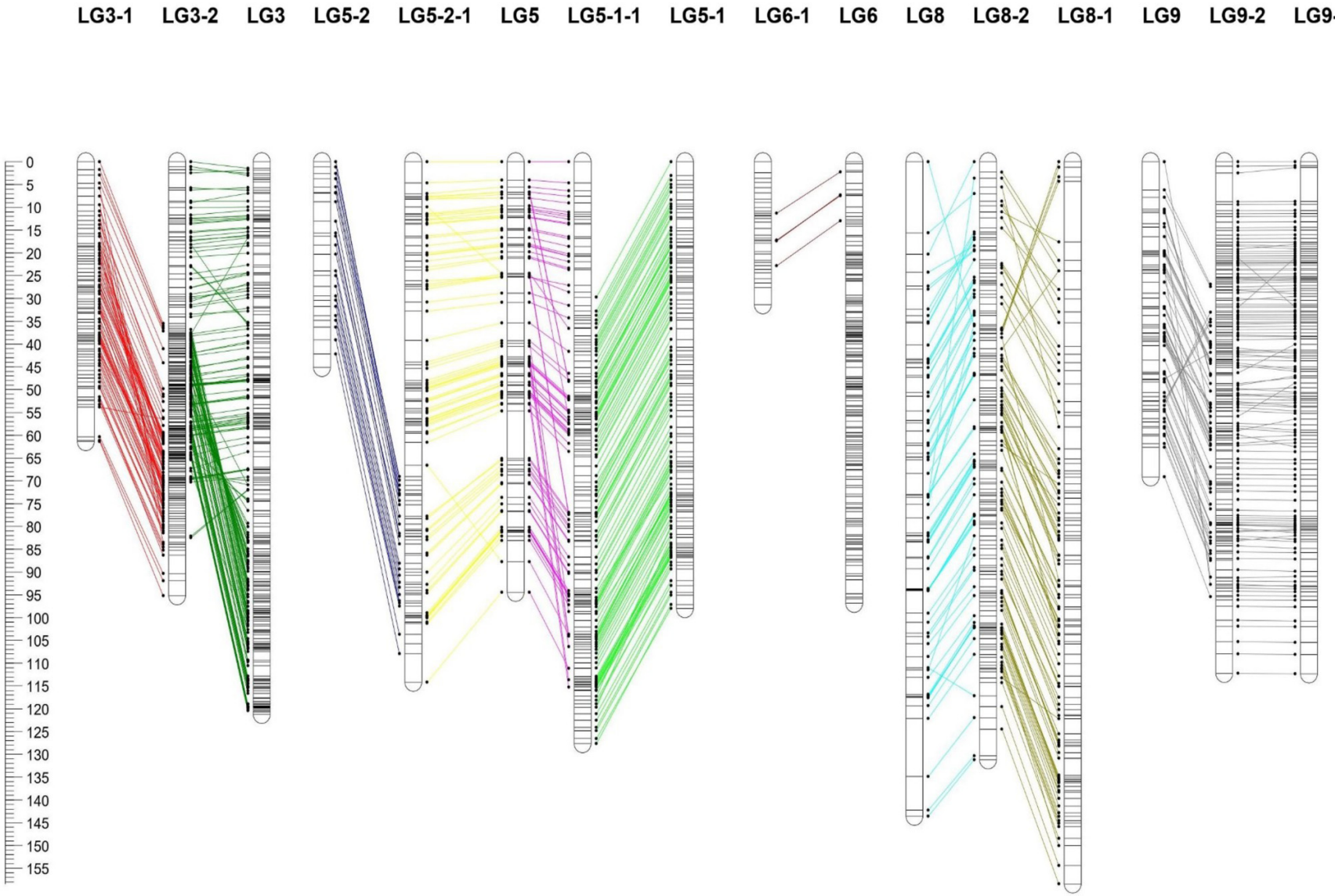


**Table 2.** Assignment, locus number, length, and marker interval of *Cynodon transvaalensis* original linkage groups (LGs) and their enriched derivatives.

| LG* | Total loci | Total length (cM) | Marker interval (cM) | Gap (>15 cM) |
|---|---|---|---|---|
| LG 1 | 230 | 119.57 | 0.52 | 0 |
| LG 2 | 98 | 89.27 | 0.91 | 0 |
| LG 3 | 267 | 121.24 | 0.45 | 0 |
| LG 3-1 | 77 | 61.38 | 0.80 | 0 |
| LG 3-2 | 251 | 95.23 | 0.38 | 0 |
| LG 4 | 157 | 100.33 | 0.64 | 0 |
| LG 5 | 77 | 94.43 | 1.23 | 0 |
| LG 5-1 | 113 | 98.01 | 0.87 | 0 |
| LG 5-2 | 25 | 45.10 | 1.80 | 0 |
| LG 5-1-1 | 179 | 127.62 | 0.71 | 0 |
| LG 5-2-1 | 101 | 114.20 | 1.13 | 0 |
| LG 6 | 188 | 96.88 | 0.52 | 0 |
| LG 6-1 | 34 | 31.33 | 0.92 | 0 |
| LG 7 | 130 | 47.91 | 0.37 | 0 |
| LG 8 | 70 | 143.60 | 2.05 | 1 |
| LG 8-1 | 98 | 158.41 | 1.62 | 0 |
| LG 8-2 | 162 | 131.21 | 0.81 | 0 |
| LG 9 | 61 | 69.11 | 1.13 | 0 |
| LG 9-1 | 132 | 112.34 | 0.85 | 0 |
| LG 9-2 | 185 | 112.22 | 0.61 | 0 |

*LG 3 and its derivatives (3-1 and 3-2), LG 5 and its derivatives (5-1 and 5-1-1, 5-2, and 5-2-1), LG 6 and its derivative 6-1, LG 8 and its derivative (8-1 and 8-2), and LG 9 and its derivative (9-1 and 9-2) revealed chromosomal rearrangements of *C. transvaalensis*.



Given the computational limitation of JoinMap 5.0, the third marker of every three SNPs in LG3 was manually removed to keep the total marker number of the combined LG under 300. With a stringent LOD score of 24, markers from LGs 3 and 3-1 were successfully grouped into a new LG, named LG 3-2 (Fig. 2; Table 2). Markers of LG 3-1 were mapped on one arm and the central part of LG 3-2 (Fig. 2). However, markers of LGs 6 and 6-1 were unable to group into a composite one due to insufficient linkage.

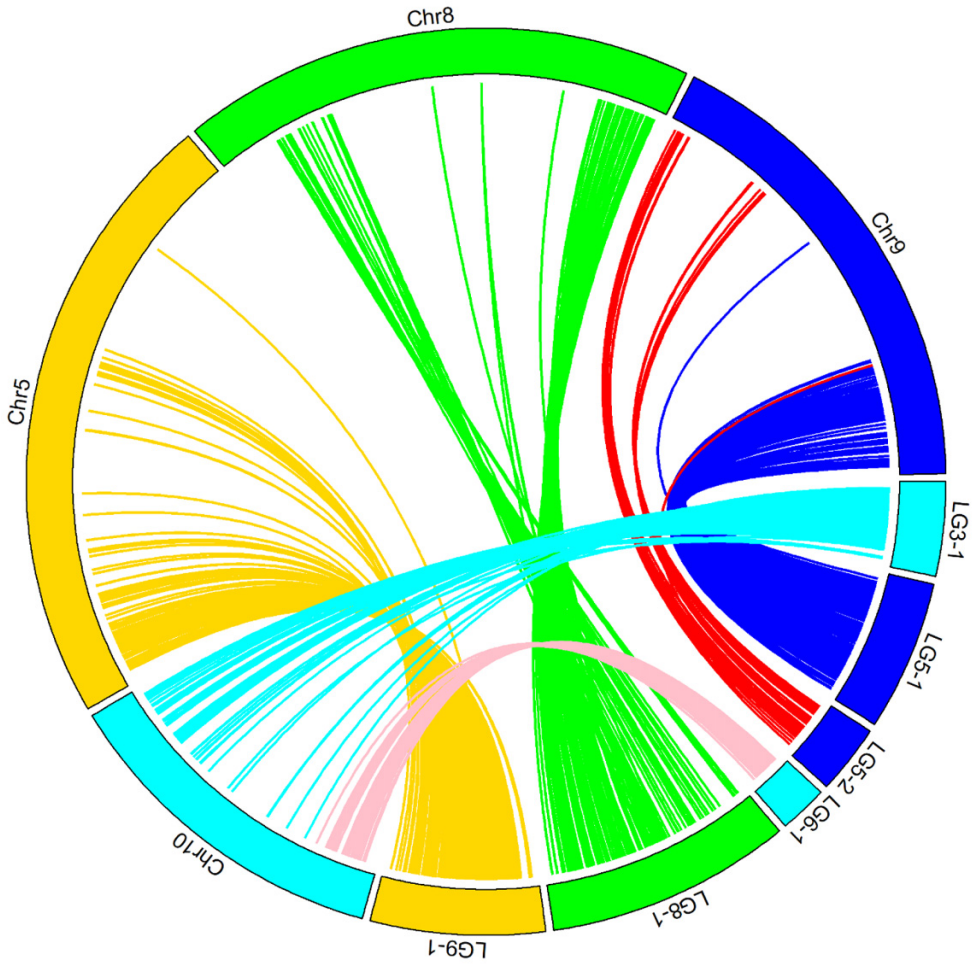
*Cynodon transvaalensis* LGs 5, 8, and 9 showed collinearity with *Oropetium thomaeum* chromosomes 9, 8, and 5, respectively. Albeit obvious, numbers of aligned markers were limited (Fig. 1). To find out possible reasons that caused low alignments of LGs 5, 8, and 9 to respective *Oropetium thomaeum* chromosomes (Fig. 1), we further investigated all the raw SNPs that aligned to Ot5, Ot8, and Ot9 and then constructed new LGs. Among the 94 909 raw SNPs, 403, 329, and 416 were aligned to Ot5, Ot8, and Ot9, respectively. These SNPs were initially filtered out due to more than 5% missing data in the mapping population or less than 6× total reading depth in individual genotypes (Yu et al. 2021). Using the LOD score of 20, 132 SNPs aligned to Ot5 were grouped into a new LG named LG 9-1, and 98 SNPs aligned to Ot8 were grouped into a new LG named LG 8-1 (Table 2; Tables S3 and S4). The SNPs aligned to Ot9 were grouped into two LGs using an LOD score of 25, named LGs 5-1 and 5-2, consisting of 113 and 25 SNPs, respectively (Table 2; Table S5). Being consistent with the initial comparative mapping results, LG 9-1 was aligned to one arm of Ot5 (Fig. 3). Meanwhile, SNPs from LGs 9 and 9-1 were pooled together to develop a composite LG named LG 9-2, the marker order of which remained largely linear with LGs 9 and 9-1 except for a few minor marker order changes (Fig. 2). The SNPs on LG8-1 were aligned onto the two arms of Ot8 (Fig. 3). SNPs from LGs 8 and 8-1 were pooled together and a composite LG 8-2 was constructed (Fig. 2). The marker order of the new composite LG 8-2 and those of its two components, LG 8 and LG 8-1, were largely collinear except for a few minor marker order changes (Fig. 2). The SNPs on LG 5-1 were aligned to one arm of Ot9, while those of LG 5-2 were aligned to another arm of Ot9 (Fig. 3). In the subsequent linkage analysis, LGs 5 and 5-1 were merged to develop a composite LG 5-1-1. Similarly, LGs 5 and 5-2 were grouped to form a composite LG 5-2-1 (Fig. 2). Thus, markers of these two composite LGs, 5-1-1 and 5-2-1, greatly enriched genomic synteny to the whole Ot9 (Fig. 3).

## Discussion

The *Cynodon* genus consists of eight species, from diploid to hexaploid, with a base chromosome number of 9 (Forbes and Burton 1963; Harlan et al. 1970*a*). The initial ancestor of *Cynodon* would be a diploid with 2n = 2x = 18 chromosomes. How the diploid evolved to have a haploid of 9 chromosomes is unknown. In the present study, we compared linkage-mapped sequences of a diploid *C. transvaalensis* (2n = 2x = 18) with genomic sequences of *Oropetium thomaeum* (2n = 2x = 20). It was evident that *C. transvaalensis* shared a high collinearity relationship with *Oropetium thomaeum* due to their close phylogenetic relationship within the same subtribe Eleusininae of the Cynodonteae tribe (Soreng et al. 2015). Two major interchromosomal rearrangement events (ρ1–ρ12–ρ1 and ρ6–ρ12–ρ6) were observed from the comparison between genomic sequences of *C. transvaalensis* and *Oropetium thomaeum* and confirmed by linkage analysis using original and derivative LGs, providing insight for understanding the 10-to-9 chromosome reduction in *C. transvaalensis*.

Comparing genome sequences of *C. transvaalensis* and *Zoysia japonica*, a recent study reported only one interchromosomal rearrangement event (Cui et al. 2021). Ancestor chromosomes corresponding to *Z. japonica* chromosomes Zj8 and Zj3 fused together, forming *C. transvaalensis* chromosome 3. Based on the comparison between *Z. japonica* and *Oryza sativa* genomes, Zj8 and Zj3 corresponded to *Oryza sativa* chromosome Os12 (ρ12) and Os1 (ρ1), respectively, suggesting only a ρ1–ρ12–ρ1 event in the genome evolution of *C. transvaalensis.* Therefore, the comparison of African bermudagrass mapped sequences with those of *Oropetium thomaeum* in this study provided novel information for understanding the genome evolution of *C. transvaalensis*. Our previous study revealed that the long arm of Ot10 is syntenic with *Oryza sativa* chromosome Os12 (ρ12), the short arm of Ot10 is syntenic with Os6 (ρ6), and Ot3 is syntenic with Os1 (ρ1) (Fang et al. 2020). *Cynodon transvaalensis* LG3 is largely syntenic to Ot3 (ρ1), with the centromeric region syntenic to the long arm of Ot10 (ρ12) in an inverse manner (Fig. 1), suggesting the ρ1–ρ12–ρ1 event in *C. transvaalensis*'s genomic evolution. Meanwhile, LG6 of *C. transvaalensis*

**Fig. 3.** SNP marker positions between *Oropetium thomaeum* (Ot) chromosome (Chr) 5 and *C. transvaalensis* (Ct) LG 9-1, between Ot Chr 8 and Ct LG 8-1, between Ot Chr 9 and Ct LG 5-1 and LG 5-2, and between Ot Chr 10 and Ct LG 3-1 and LG 6-1. *Oropetium thomaeum* chromosomes and *C. transvaalensis* LGs are oriented clockwise.






is largely syntenic to Ot2 (ρ6) and partially aligned to the short arm of Ot10 (ρ12) (Figs. 1 and 3). In subsequent linkage analyses, markers from LG 3-1 derived from the long arm of Ot10 (ρ12) fused into the end of the *C. transvaalensis* LG 3 (Fig. 2). The results of this study are consistent with findings reported by Fang et al. (2020) that ρ12 inserted into two pairs of homologous *C. dactylon* LGs corresponded to ρ1 and ρ6. However, a new revelation was added in elucidating the interchromosomal rearrangement pattern that ρ12 fuses with ρ1, reinforcing the NCF ρ1–ρ12–ρ1 in *Cynodon*. The similar genomic events between *C. dactylon* and *C. transvaalensis* are consistent with the close relationship between these two species, supporting the finding that the chromosome reduction from 10 to 9 relative to *Oropetium thomaeum* happened before the allotetraploid *C. dactylon* was formed (Fang et al. 2020). The present analysis further indicated that the genomic events resulting in the chromosome number reduction from 10 to 9 in the *Cynodon* genus occurred before the speciation of *C. transvaalensis*. It was likely that the genomic events led to the initial diploid ancestor, which evolved over time into several diploid species, including *C. transvaalensis*, *C. dactylon* var. *aridus* J.R. Harlan & de Wet, *Cynodon barberi* Rang. & Tadul., *Cynodon plectostachyus* (K. Schum.) Pilger, and *Cynodon incompletus* var. *incompletus* (Harlan et al. 1970*c*). Up to now, the two diploid species forming the allotetraploid *C. dactylon* are unknown. The allotetraploid *C. dactylon* likely evolved from the hybridization of two unknown but different diploid species, and then the chromosomes doubled. Similar findings were also reported for the genus *Miscanthus* of the Panicoideae subfamily. Two primary species used for interspecific hybrid development and biomass bioenergy production, *Miscanthus sinensis* Andersson and *Miscanthus sacchariflorus* (Maxim.) Franch., both showed similar genomic synteny against sorghum (*Sorghum bicolor* (L.) Moench) (Swaminathan et al. 2012; Dong et al. 2018; Mitros et al. 2020). Interchromosomal rearrangement being involved with telomeric regions of invaded and invading chromosomes was a common chromosome number reduction mechanism in the grass family (Luo et al. 2009; X. Wang et al. 2015).

The LGs 5 and 9 of *C. transvaalensis* were partially aligned to Ot9 and Ot5, respectively (Fig. 1). The subsequent linkage analyses confirmed that LG 9 was only aligned to one half of Ot5. This result is consistent with the results related to Ot5 in *C. dactylon* (Fang et al. 2020). By adding more SNP sequences,

we were able to identify both arms of Ot9 linked to the LG 5 of *C. transvaalensis* (Fig. 3). Another chromosome rearrangement event observed was on *C. transvaalensis* LG 7. The full length of *C. transvaalensis* LG 7 was aligned to a large section of Ot6, suggesting either a noticeable deletion may have happened in Ot6 or LG 7 may not cover the *C. transvaalensis* chromosome. Similarly, Fang et al. (2020) also found part of Ot6 aligned to *C. dactylon* LGs 11 and 12. In the initial comparative mapping, *C. transvaalensis* LG 8 was aligned to Ot8 with two sequences. Later, we demonstrated the correspondence between Ot8 and *C. transvaalensis* LG 8 with more SNP sequences (Fig. 3). Although derivative LGs were constructed using SNPs without filtering, these SNPs aligned to the *Oropetium thomaeum* reference genome and the orders of SNPs in derivative LGs corresponded well to their physical locations on the *Oropetium thomaeum* reference genome (Fig. 3). Therefore, this method can reveal hidden genomics information that comparative mapping analysis cannot provide and was successfully used in a study conducted by Fang et al. (2020).

Several studies have investigated the major chromosome rearrangement patterns during the evolution of species in Chloridoideae. Comparative genomic analyses between *Oryza sativa* and *Zoysia* spp. revealed that two NCF events, ρ2–ρ10–ρ2 and ρ6–ρ9–ρ6, occurred in the process from the x = 12 ancestor to the x = 10 ancestor (F. Wang et al. 2015; Huang et al. 2016). These two NCFs have also been observed in other Chloridoideae species, such as finger millet (*Eleusine coracana* (L.) Gaertn) (Srinivasachary et al. 2007) and *C. dactylon* (Fang et al. 2020). In addition, the paleoancestor chromosomes ρ9 and ρ10 played a role as invading chromosomes in the NCFs found in Panicoideae species such as *Sorghum bicolor* (Paterson et al. 2009) and foxtail millet (*Setaria italica* (L.) P. Beauvois) (Zhang et al. 2012). In the genome comparison between *Oryza sativa* and *Oropetium thomaeum*, Fang et al. (2020) showed that Ot1 was derived from ρ2–ρ10–ρ2 and Ot2 from ρ6–ρ9–ρ6. Our results indicated that *C. transvaalensis* LG 1 corresponded to Ot1 and *C. transvaalensis* LG 6 to Ot2, further confirming NCFs ρ2–ρ10–ρ2 and ρ6–ρ9–ρ6 in *C. transvaalensis*. Khanal et al. (2017) observed three independent translocation events (i.e., Os3 + Os4, Os1 + Os12, and Os11 + Os12) in *C. transvaalensis* apart from the two NCFs shared with *Zoysia* spp. The ρ12 played a vital role in the chromosome number reduction from x = 10 to x = 9 in *E. coracana*, *C. dactylon*, and *C. transvaalensis* in the Chloridoideae subfamily, and *Setaria italica* and St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze) in the Panicoideae subfamily (Srinivasachary et al. 2007; Zhang et al. 2012; Yu et al. 2018). Instead of invading ρ5 as reported for *E. coracana*, *Setaria italica*, and *Stenotaphrum secundatum*, one part of ρ12 invading ρ1 and another part of ρ12 invading ρ6 is unique and has only been identified in the *Cynodon* genus so far by Fang et al. (2020) and confirmed in this study.

## Conclusions

*Cynodon transvaalensis* is an important diploid species. Our comparative analysis between sequences mapped in *C. transvaalensis* and those of *Oropetium thomaeum* indicated that two interchromosomal rearrangement events resulted in the chromosome number reduction from 10 to 9. The chromosomal reduction likely created the initial ancestor of the *Cynodon* genus as both *C. transvaalensis* (2x = 18) and *C. dactylon* (4x = 36) possess the genomic characteristics. The findings fill a knowledge gap that the whole chromosomal reduction played a key role in the initiation of *Cynodon*. The findings also provide an improved basis for accurate genome assembly in *C. transvaalensis* and future orthologous functional gene cloning.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### Data availability

Supplemental tables and bioinformatic scripts are available in the supplemental data.

## Author information

### Author contributions

SHY conducted the experiments, analyzed the data, and wrote the manuscript. HXD and TLF helped in data analyses and revised the manuscript. YQW conceived the project, created the population, and revised the manuscript before submission.

### Competing interests

The authors have declared that no competing interests exist.


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## Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/gen-2021-0122.

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