Cytogenetic studies in the genus *Tribolium* (Poaceae: Danthonieae).

IV. Section *Uniolae*

Nerina C. Visser and J.J. Spies*

Department of Botany and Genetics, University of the Orange Free State, P.O. Box 339, Bloemfontein, 9300, Republic of South Africa

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The section *Uniolae* of the genus *Tribolium* Desv. comprises four species, namely *T. alternans* (Nees) Renv., *T. amplexum* (Nees) Renv., *T. brachystachyum* (Nees) Renv. and *T. uniolae* (L. f.) Renv. *Tribolium alternans*, *T. amplexum* and *T. uniolae* are morphologically similar and form a hybrid swarm. Thus, only two species should be recognized, namely *T. brachystachyum* and a *T. uniolae* hybrid swarm. Diploid, tetraploid and hexaploid specimens have been observed in the *T. uniolae* hybrid swarm, whereas all the *T. brachystachyum* specimens were tetraploid. Segmental allopolyploidy, tending towards allopolyploidy, is present in both species.

Die seksie *Uniolae* van die genus *Tribolium* Desv. bestaan uit vier spesies, naamlik *T. alternans* (Nees) Renv., *T. amplexum* (Nees) Renv., *T. brachystachyum* (Nees) Renv. en *T. uniolae* (L. f.) Renv. *Tribolium alternans*, *T. amplexum* en *T. uniolae* stem morfologies ooreen en vorm 'n basterkompleks. Slegs twee spesies behoort dus erken te word, naamlik *T. brachystachyum* en 'n *T. uniolae* basterkompleks. Diploëde, tetraploëde en heksaploëde eksemplare is by die *T. uniolae* basterkompleks waargeneem. terwyl die tetraploëde eksemplare by *T. brachystachyum* gevind is. Segmentele allopolyploëdie, wat neig na allopolyploëdie, kom by beide spesies voor.

**Keywords:** Cytogenetics, morphology, Poaceae, *Tribolium*.

* To whom correspondence should be addressed.

**Introduction**

The genus *Tribolium* Desv. comprises twelve species and is subdivided into three sections (Visser & Spies 1994a), of which only the section *Uniolae* will be discussed in this paper. *Tribolium alternans* (Nees) Renv. is distributed in sandy soil, in river flats in the south-western Cape Province, whereas *T. amplexum* (Nees) Renv. is distributed in disturbed sandy soil in the same area (Gibbs Russell et al. 1990). *Tribolium brachystachyum* (Nees) Renv. is restricted to the mountains between Paarl and Franschoek. *Tribolium uniolae* (L. f.) Renv. is widely distributed and grows from the eastern extremity (Port Elizabeth) of the distribution area of the genus to the western extremity, the Atlantic Ocean (Gibbs Russell et al. 1990).

Cytogenetic studies on *Tribolium* suggest a basic chromosome number of six (Spies et al. 1992; Visser & Spies 1994 a–c) or a basic chromosome number of seven reported by De Wet (1960), based on a *T. uniolae* specimen with \(2n = 28\). We attribute the deviation to the possible presence of B-chromosomes in De Wet’s (1960) specimen, since no similar deviation was observed in any of the more than 200 specimens studied in our laboratories.

The meiotic chromosome behaviour of the species in the section *Uniolae* was studied in order to determine the type of ploidy present among the different species, as the type of ploidy present indicates whether hybridization played any role during the development of the different species in this section. In addition to the cytogenetic data, morphological data was included in an attempt to determine the phylogenetic relationships among the various species in the section *Uniolae*.

**Materials and Methods**

The materials used were collected in the field. Voucher herbarium specimens are housed in the Geo Potts Herbarium (BLFU), Department of Botany and Genetics, University of the Orange Free State, Bloemfontein, and the National Herbarium (PRE), Pretoria. These specimens and their localities are listed in Table 1. The methods used during this study included meiotic analyses and factor analyses of morphological attributes (Visser & Spies 1994c).

Chiasma frequencies are regarded as the average number of chiasmata per bivalent and were calculated as the total number of chiasmata per cell divided by the haploid chromosome number of the cell. This method was used because it is congruous to other calculations done, especially with determining genome homology within an individual (Kimber & Alonso 1981).

**Results**

Four *T. alternans* specimens, 12 of *T. amplexum*, 11 of *T. brachystachyum* and 87 of *T. uniolae* were studied morphologically. Twenty-one morphological characters of each specimen were studied [listed elsewhere (Visser & Spies 1994a)]. All the species in the section *Uniolae* are perennial, tufted grasses, growing to a maximum of 700 mm. The inflorescences form compact spikes. The spikelets are distichously arranged, 4 – 7 mm long and are 4 – 9-flowered.

The glumes of *T. alternans*, *T. amplexum* and *T. uniolae* specimens are glabrous to sparsely pubescent, whereas the glumes of *T. brachystachyum* are densely pubescent. The glumes of herbarium specimens of *T. brachystachyum* and *T. uniolae* in the section *Uniolae* were studied under an electron microscope. All the species in this section possess two types of trichomes on the spikelet, namely large trichomes on the glumes and a smaller fringe of trichomes on the lemma. The large trichomes are long, glassy and taper off apically, whereas the shape of the smaller trichomes differ between the various species (Visser & Spies 1994a). *Tribolium alternans*, *T. amplexum* and *T. brachystachyum* possess club-shaped trichomes, whereas it varies from tapered to club-shaped in *T. uniolae* (Visser & Spies 1994a).

Factor analyses of the morphological data revealed that *T. alternans*, *T. amplexum* and *T. brachystachyum* differ morphologically (Figure 1). However, *T. uniolae* overlaps to such an extent with both *T. alternans* and *T. amplexum*, that morphological separation of these three species is impossible. All the
swarm as a complex mixture of parental forms, hybrids, back­
chronized second divisions (Figure 2J) and uneven segregation
chromosome segregation during metaphase I and II (Figure 2G), various anaphase I and II bridges (Figure 2H, I), unsyn­
abnormalities include univalents during metaphase I (Figure
ai.
hybrid swarm. We regard a hybrid
part of a
Tribolium brachystachyum, as
we recognize only two species in the section
T. brachystachyum, (n = 12 + 0 - 4B) and a
T. unioiae (n = 6 + 0 - 1B; n = 12 + 0 - 3B; n = 18 + 0 - 4B)
—3119 (Calvinia): 41 km from Vanrhynsdorp to Nieuwoudtville on
the summit of Vanrhyns Pass (–AC), Spies 3462 (n = 12); Klawer/Elizabetehoekfontein intersection on the Nieuwoudtville road
(–CD), Spies 4987* (n = 12).
—3218 (Clanwilliam): Versfeld Pass (–DD), Spies 4585 (n = 6 + 0 – 1B), Spies 5008 (n = 12), Spies 5009 (n = 12), Spies 5010 (n = 12) & Spies 5011 (n = 18).
—3318 (Cape Town): 2 km east of Manne (–CB), Spies 5012* (n = 18); 18 km from Wellington to Worcester via Vanrhynsdorp (–DB), Spies 4440 (n = 12).
—3319 (Worcester): 14 km from Wellington to Wolseley (–CA), Davidsde 33926a (n = 12); Du Toit’s Kloof Pass (–CA), Spies 4447 (n = 12), Spies 4449 (n = 12), Spies 4609 (n = 12 + 0 - 2B), Spies 5015 (n = 12 + 0 – 2B), Spies 5016 (n = 12 + 0 – 2B), Spies 5017 & Spies 5025* (n = 2 + 0 – 2B); Franschhoek Pass (–CC), Davidsde 33859 (n = 12 + 0 – 2B), Davidsde 33879 (n = 18), Spies 4589 (n = 12 + 0 – 2B), Spies 4590 (n = 12 + 0 – 2B), Spies 4591 (n = 12 + 0 – 1B), Spies 5024 (n = 12 + 0 – 1B), Spies 5026 (n = 12), Spies 5029 (n = 12 + 0 – 2B) & Spies 5030* (n = 12 + 0 – 1B); 4 km from Villiersdorp to Worcester (–CD), Davidsde 34123 (n = 12 + 0 – 2B).
—3418 (Simonstown): Sir Lowry’s Pass (–BB), Spies 4612 (n = 12 + 0 – 2B).
—3419 (Caledon): 8 km from Botriver to Villiersdorp (–AA), Davidsde 33839* (n = 18 + 0 – 4B); Vlijoen’s Pass (–AA), Spies 4652 (n = 12); Shaw’s Pass (–AD), Davidsde 34114 (n = 12 + 0 – 1B); Akkedisberg Pass between Stanford and Rivieronderend (–BC), Davidsde 34109 (n = 12); 37 km from Caledon to Bredasdorp (–DB), Spies 3531* (n = 12).
—3420 (Bredasdorp): 1 km from De Hoop Nature Reserve (–AD), Spies 4625 (n = 12) & Spies 5052 (n = 12); Elim turn-off at Bredasdorp (–CA), Spies 3536 (n = 12).

| Voucher specimen | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|------------------|---|---|---|---|---|---|---|---|---|
| Spies 3875       | 12| 1.5| 0–2| 5.9| 4.7| 0.7| 40| 10| 60|
| Spies 3893       | 12| 1.5| 0–2| 5.8| 4.8| 0.7| 30| 80| 36|
| Average          | —| 1.5| —| 5.85| 4.75| 0.7| 60| 20| 63|

T. unioiae

Table 1 List of species cytotogenetically studied, voucher specimen numbers and localities according to the degree reference system (Edwards & Leistner 1971)

| Voucher specimen | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|------------------|---|---|---|---|---|---|---|---|---|
| Davidsde 33839   | 18| 1.31| 0–3| 13.3| 4.3| 0.2| 20| 0| 0|
| Davidsde 33926   | 12| 1.46| 0| 6.5| 3.17| 1.17| 0| 10| 75|
| Davidsde 34109   | 12| 1.23| 0| 9.2| 2.1| 0.4| 0| 40| 30|
| Davidsde 34114   | 12| 1.35| 0–1| 7.8| 2.2| 1| 0| 80| 20|
| Davidsde 34123   | 12| 1.34| 0–2| 7.9| 1.7| 1.2| 0| 75| 0|
| Spies 4447       | 12| 1.44| 0| 6.4| 3.3| 1| 0| 15| 20|
| Spies 4449       | 12| 1.34| 0| 7.9| 2.3| 0.9| 10| 13| 20|
| Spies 4589       | 12| 1.64| 0–2| 4.7| 2.6| 4.7| 20| 0| 10|
| Spies 4609       | 12| 1.48| 0–2| 6| 3.7| 2.3| 10| 10| 10|
| Spies 4612       | 12| 1.47| 0–2| 6.2| 2.4| 3.4| 44| 0| 10|
| Average          | 12| 1.42| —| 6.9| 2.6| 1.8| 10| 27| 24|
| Average          | 18| 1.31| —| 13.3| 4.3| 0.2| 20| 0| 0|

* haploid chromosome number, number of B-chromosomes, chiasma frequencies, chromosome configurations during diakinesis, and meiotic irregularities. Only specimens where twenty or more cells of the relevant meiotic stages were studied, are listed.

Table 2 Meiotic analysis of Tribolium brachystachyum and T. unioiae specimens

Chromosomes were described as B-chromosomes when their number deviated from the expected chromosome number and their meiotic chromosome behaviour deviated from the expected behaviour of additional aneuploid chromosomes. The behaviour of B-chromosomes in Tribolium was discussed by Spies et al. (1992). It is impossible to recognize B-chromosomes in all cells of a specimen. However, when certain meiotic stages or certain cells of a particular meiotic stage indicate the presence of B-chromosomes without doubt, chromosomes deviating from the expected behaviour in other cells of the same specimen were regarded as being B-chromosomes. This was only done where the 'abnormal' chromosome behaviour did not occur in any specimen without any B-chromosomes.

The genome homology of two T. brachystachyum specimens have been determined according to the models proposed by Kimber and Alonso (1981). The genome analyses indicated that the observed chromosome configurations corresponded best with the expected frequencies for the 2.2-model, with x-values of 1 (Table 3). The 2.2-model indicates that two sets of genomes are present. Each set consists of two genomes and the relative similarity of the genomes in a set is considered to be 0.5. The relative similarity between the sets of genomes is expressed by an x-value that may vary between 0.5 (differences between sets are similar to differences within a set) and 1 (sets differ very much). The x-values for the T. brachystachyum specimens was 1 (Table 3), thus indicating no homology between the two sets of genomes. Based on the species used during this study, this species is an alloploid species.
Figure 1  Factor analysis of specimens representative for section Uniolae.  A. Factors 1 and 2.  B. Factors 1 and 3.  C. Factors 2 and 3.  ▲, T. alternans; □, T. amplexum; ●, T. brachystachyum; O, T. uniolae.  1, Average values for T. alternans; 2, Esterhuizen 585; 3, Garside 1331; 4, Sandwith 180; 5, Taylor 5585; 6, average values for T. amplexum; 7, Barker 693; 8, Boucher 575; 9, Smook 3066B; 10, Strey 490; 11, average values for T. brachystachyum; 12, Davidse 34142; 13, Esterhuizen 2855; 14, Spies 3875; 15, Spies 4611; 16, average values for T. uniolae; 17, Davidse 33488; 18, Davidse 33742; 19, Spies 4417; 20, Spies 4585.
The *T. uniolae* hybrid swarm was cytogenetically represented by 36 specimens (Table 1). Polyploidy was frequently encountered (Figure 3A–F). One specimen was diploid (*n* = *x* = 6) (Figure 3A), 31 were tetraploid (*n* = 2*x* = 12) (Figure 3B) and four were hexaploid (*n* = 3*x* = 18) (Figure 3C). Chiasma frequencies vary within a particular polyploid level (Table 2), with averages for the tetraploids and hexaploids being 1.42 and 1.31, respectively. Chromosome configurations vary during diakinesis and include multivalents (Figure 3B,E) (Table 2).
Various meiotic chromosome abnormalities were observed. These abnormalities included the following: univalents (or B-chromosomes) during metaphase I (Figure 3E), chromosome laggards during anaphase I (Table 2), micronuclei during telophase I and II (Table 2), precocious segregation of one bivalent on the metaphase plate, anaphase bridges (Table 2), uneven segregation of chromosomes during anaphase I (Figure 3F) and cell fusion.

Nought to four B-chromosomes are present in the specimens from the *T. uniolae* hybrid swarm (Table 1). The number of B-chromosomes varies per specimen and per polyploid level. The B-chromosomes form univalents during metaphase (Figure 3E), which are either on the metaphase plate or away from it. The B-chromosomes on the metaphase plate form laggards during anaphase I that result in micronuclei during telophase I.

Genome analyses of nine specimens revealed that the 2:2-model of Kimber and Alonso (1981) fitted the specimens to the greatest degree, with x-values of 1 or approximately 1 in all specimens, except *Spies 4589* (x-value = 0.65) and *Spies 4612* (x-value = 0.75) (Table 3). With the exception of these two segmental allopolyploid specimens, all other specimens were allopolyploids.

**Discussion**

The species of the genus *Tribolium* exhibit great genetical and morphological variation (*Spies et al. 1992*). This variability within a species is particularly evident in *T. uniolae* in the section *Uniolae*. This perennial species is a tufted grass, varying in length from 100 to 600 mm. The lengths of the inflorescences vary from 8 to 70 mm and are often branched at the base. The degree of hairiness differs extensively. Specimens can either be glabrous or hairy on the stems and/or the leaves and/or the panicle. Specimens with all these combinations of characters have been observed.

Due to the wide range of morphological variation in *T. uniolae*, this species includes many characters associated with *T. alternans*, *T. amplexum* and *T. brachystachyum*. The species in this section are morphologically similar, although a few mor-

### Table 3 Genomic relationships in the tetraploid *T. brachystachyum* and *T. uniolae* specimens according to the models of Kimber and Alonso (1981)*

|        | 4:0 | 3:1 | 2:2 | 2:1:1 |
|--------|-----|-----|-----|-------|
| *T. brachystachyum* |     |     |     |       |
| Spies 3875 | (4.74) | 0.5 (4.98) | 1 (1.48) | 0.92 (2.97) |
| Spies 3893 | (4.7)  | 0.5 (4.93) | 1 (1.47)  | 0.92 (2.94)  |
| *T. uniolae* |     |     |     |       |
| Davidse 33926a | (4.77) | 0.5 (4.97) | 0.99 (2.99) | 0.85 (4.21) |
| Davidse 34109 | (10.11) | 0.5 (10.33) | 1 (5.16) | 0.75 (10.01) |
| Davidse 34114 | (6.89) | 0.5 (7.09) | 0.99 (4.09) | 0.84 (6.7) |
| Davidse 34123 | (7.17) | 0.5 (7.36) | 1 (4.32) | 0.76 (7.13) |
| Spies 4440 | (4.46) | 0.5 (4.66) | 0.98 (2.42) | 0.84 (3.81) |
| Spies 4449 | (7) | 0.5 (7.21) | 1 (3.97) | 0.82 (6.78) |
| Spies 4589 | (2.3) | 0.5 (2.43) | 0.65 (2.22) | 0.74 (2.27) |
| Spies 4609 | (4.23) | 0.5 (4.44) | 0.93 (2.46) | 0.9 (3.42) |
| Spies 4612 | (4.21) | 0.5 (4.39) | 0.75 (3.71) | 0.79 (4.08) |

* The x-value is followed by the sum of squares of the deviation between the observed and the expected values for each model, in parentheses.
phological differences have been observed between *T. uniolae* and *T. brachystachyum*. The number of trichomes on the glumes varies for *T. uniolae*, whereas for *T. brachystachyum*, they are always dense. The structure of the trichomes on the glumes corresponds for the two species (Visser & Spies 1994a). Both species have club-shaped trichomes at the base and on the edge of the lemma (Visser & Spies 1994a), but the degree of hairiness between the *T. uniolae* specimens differs. *Tribolium brachystachyum* is very setaceous and contains trichomes on the leaves, the blades and the inflorescences, whereas *T. uniolae* is glabrous or setaceous in any combination of these three parts. Morphologically, *T. uniolae* varied for every character examined, except that none of the specimens had trichomes on the stems of the plants.

The morphological overlap between the species in this section, the large morphological variation observed in *T. uniolae*, the presence of sexually reproducing specimens, facultative apomixis (Visser & Spies 1994b) and the degree of variation in polyploid levels observed for the southern Cape, found in this geographically isolated location, making this area an ideal location for studying evolution in the species.

The genomic constitution of the various polyploid specimens were studied with the use of Kimber and Alonso's (1981) models. *Tribolium brachystachyum* has been classified as a segmental allotetraploid species, tending towards allopolyploidy (Table 3). However, the presence of quadrivalents indicates that the genomes of this species must, to some extent, be similar.

The genomic constitution of the various *T. uniolae* specimens differed. The 2:2-model fitted the specimens to the greatest degree (Table 3), with x-values of 1 or tending towards 1. The only exceptions were *Spies 4589* (x-value = 0.65) and *4612* (x-value = 0.75). Both these specimens have a very high number of quadrivalents per cell in comparison to all the other specimens (Table 2), thus indicating a greater degree of genome homology in these specimens. Nevertheless, all specimens are either allopolyploid or segmental allopolyploids and hybridization in all of them is, therefore, implied. The allopolyploid and segmental allopolyploid origin indicates that hybridization played an important part in the evolution of the section *Uniolae*.

*Tribolium uniolae* is usually found in mountainous areas. Five specimens were collected on Piekberg (*Spies 4585, 5008, 5009, 5010 & 5011*). Due to the absence of other mountain ranges that link this locality to the mountain ranges in the southern Cape, Piekberg is geographically isolated. Three different polyploid levels have been observed in these specimens, namely diploid (*Spies 4585*), tetraploid (*Spies 5008, 5009 & 5010*) and hexaploid (*Spies 5011*). The whole known range of polyploid levels observed for *T. uniolae* is, therefore, found in this geographically isolated location, making this area an ideal location for studying evolution in the species.

Morphologically, the five specimens differed. The prostrate diploid specimen is morphologically similar to one of the tetraploid specimens (*Spies 5010*). Both specimens are relatively small and tender, lack trichomes on the inflorescences and have curly leaves. The other tetraploid specimens (*Spies 5008 & 5009*) correspond with the hexaploid specimen (*Spies 5011*), except for the trichomes on the latter specimen's inflorescences. The increasing morphological variation at higher ploidy levels supports a hybrid origin for these specimens.

The apparent ability of *T. alternans*, *T. amplexum*, *T. brachystachyum* and *T. uniolae* to hybridize and to exploit the advantages of hybrid species complexes or hybrid swarms, with ranges of chromosome numbers and genomes, is ancient in the grasses (De Wet 1986). Natural hybridization is common in the grasses and the variability within a hybrid population increases (Ehrendorfer 1980). This level of genetic variability allows the grasses to take advantage of new habitats (Ehren­ dorfer 1980), and, in the case of *Tribolium*, disturbed habitats such as road sides and agricultural ground (Spies et al. 1992).

Conclusions

The basic chromosome number of the species in the section *Uniolae* is *n* = 6. A wide range of ploidy levels have been observed in this section, namely diploid (*T. uniolae*), tetraploid (*T. brachystachyum* and *T. uniolae*) and hexaploid (*T. uniolae*). Two types of polyploidy have been identified, namely allopolyploidy (*T. brachystachyum* and *T. uniolae*) and segmental allopolyploidy (*T. uniolae*). This fact, as well as the results of the principal-component analysis, indicate that hybridization played a major role during the evolution of these species. The cyto­ genetic and morphological evidence lead to the recognition of a *T. uniolae* hybrid swarm, including the species *T. alternans*, *T. amplexum* and *T. uniolae*. It is therefore suggested that the number of species in this section should be reduced to two, namely *T. brachystachyum* and a *T. uniolae* hybrid swarm.

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