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Cytogenetic investigation of *Triticum timopheevii* (Zhuk.) Zhuk. and related species using the C-banding technique

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Abstract *Triticum timopheevii* and related species *T. militinae* ($2n = 28$, A^tG) and *T. zhukovskyi* ($2n = 42$, A^mA^tG), hybrids *T. kiharae*, *T. miguschovae*, the amphidiploid *T. timopheevii* × *T. tauschii* (all $2n = 42$, A^tGD), *T. fungicidum* (ABA^tG) and *T. timonovum* ($2n = 56$, A^tA^tGG) were analyzed using the C-banding technique. Chromosomes of the A^m and A^t genomes in the karyotype of *T. zhukovskyi* differed in their C-banding pattern. Partial substitutions of A^t-genome chromosomes and a complete substitution of the G-genome chromosomes by homoeologous chromosomes of an unidentified tetraploid wheat species with an AB genome composition were found in the *T. timonovum* karyotype. A^t- and G-genome chromosomes in the karyotypes of all studied species had similar C-banding patterns and were characterized by a low level of polymorphism. The comparative stability of the A^t and G genomes is determined by the origin and specifity of cultivation of studied species.

Key words *Triticum timopheevii* · Amphiploids · Karyotype· C-banding · Polymorphism

Introduction

Triticum timopheevii (Zhuk.) Zhuk., a tetraploid wheat with an A^tG genome composition, was first discovered by P.M. Zhukovskyi in Western Georgia. On the basis of its distinct differences from other cultivated tetraploid wheats, *T. timopheevii* was considered to be a separate species. Timopheevi wheat is cultivated at altitudes of 300–1200 m in regions with a humid climate (average rainfall per year 900–1200 mm). Its high resistance to a majority of plant pathogens attracted the attention of breeders, and it was used to create some immune common wheat varieties. At present *T. timopheevii* is no longer cultivated as an agricultural crop.

T. militinae Zhuk. et Migusch. ($2n = 28$, A^tG) is a natural mutant of *T. timopheevii* (Dorofeev et al. 1979). Very similar to *T. timopheevii* in its habitat and resistance to plant pathogens, *T. militinae* is free threshing.

T. zhukovskyi Menabde et Ericzjan was found in a Zanduri population (Georgia) together with *T. timopheevii* and *T. monococcum* L. in 1958. The investigations of some authors showed that this species resulted from the spontaneous hybridization of *T. timopheevii* with *T. monococcum* (Tavrin 1963; Upadhya and Swaminathan 1969). Bowden (1959) proposed that the genome formula for *T. zhukovskyi* is A^tA^tG. According to an alternative hypothesis, this species derived as an autotetraploid of *T. monococcum* (Menabde 1972). *T. zhukovskyi* is immune to many plant pathogens.

T. kiharae and *T. miguschovae* are artificially synthesized hexaploid wheats with the genome formulas A^tGD (Dorofeev et al. 1979; Badaeva et al. 1989, 1990a). The former was first obtained in Japan by crossing *T. timopheevii* × *T. tauschii* (Dorofeev et al. 1979), and the latter is a hybrid between *T. militinae* and *T. tauschii* ssp. *strangulata* (Zhirov 1980). Both species are similar in morphology, but *T. miguschovae* has a more intense anthocyan color. The amphidiploid (AD) *T. timopheevii* × *T. tauschii* was developed by E.R. Sears (University

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of Missouri, USA), and the seeds were kindly supplied by S.S. Maan (North Dakota State University, USA).

T. timonovum Heslot et Ferrary is an octoploid wheat obtained upon duplication of a set of *T. timopheevii* chromosomes by means of colchicine treatment. Some of the chromosomes of the A^t and one of the G genome were substituted by homoeologues of A and B genomes of an unidentified emmer wheat species (Badaeva et al. 1990a).

T. fungicidum Zhuk. is an artificially synthesized wheat (2n = 56) developed by P.M. Zhukovsky by crossing *T. timopheevii* × *T. carthlicum* Nevsk. (= *T. persicum*) with spontaneous duplication of chromosomes in the F_1 (Zhukovsky 1971). The chromosome set of *T. fungicidum* corresponded to the sum of genomes of the parental species (Badaeva et al. 1990a).

All of the species mentioned above contain chromosomes of the A^t and G genomes inherited from *T. timopheevii* or *T. militinae*. Thus, a comparison of their karyotypes makes it possible to study the influence of ploidy level and additional genomes on karyotype structure.

Material and methods

Nine accessions of *T. timopheevii*, 2 of *T. militinae* and *T. timonovum* and 1 of *T. zhukovskyi*, *T. kiharae*, *T. miguschovae*, AD *T. timopheevii* × *T. tauschii* and *T. fungicidum* were used for the investigation (Table 1).

Chromosome preparation and the C-banding technique were as described earlier (Badaev et al. 1985). For chromosome staining, Giemsa-stain (Merck, FRG and Fisher Scientific, USA) was used. Chromosomes of the A, B, A^t, A^m and G genomes were identified according to genetic nomenclature (Gill 1987; Zelenin et al. 1987; Friebe et al. 1990; Badaeva et al. 1991).

Results and discussion

Polymorphism of A^t and G-genome chromosomes in tetraploid species (*T. timopheevii* and *T. militinae*)

Our investigation of the 11 lines of tetraploid *T. timopheevii* and *T. militinae* showed that these species are characterized by high karyotype stability with respect to chromosomal aberrations, which is in agreement with published data (Zurabishvili et al. 1978; Hutchinson et al. 1982; Dvorák 1983; Kawahara 1984; Gill and Chen 1987; Shang et al. 1988; Badaeva et al. 1990b). Among the studied forms, only 2 [line 6, Table 1, and the line described by Zurabishvili et al. (1978)] possess a T 1G·5G translocation (Fig. 2, lane g). The same translocation was also found in 2 accessions of *Triticum araraticum* Jakubz. from Azerbaijan.

A comparison of the karyotypes of the 9 accessions of *T. timopheevii* (var '*typicum*' and '*viticulosum*') maintained in different collections (Table 1) shows similar C-banding patterns (Figs. 1, 2). The appearance of additional intercalary C-bands in the $4A^t$ and $7A^t$ chromosomes in the karyotype of line No. 9 (Fig. 1, lane c) was due to the use of Giemsa-Fisher for chromosome staining (for other samples Giemsa-Merck was used). C-banding pattern stability is typical for *T. timopheevii*, and it differentiates this species from its highly polymorphic wild relative, *T. araraticum* (Badaeva et al. 1990b). In general, the karyotype of *T. araraticum* contains less heterochromatin than *T. timopheevii*. The most obvious differences were for chromosomes $1A^t$, $7A^t$, 1G, 4G and 5G. That the *T. timopheevii* genome has a significantly lower variability than its wild relative, has also been detected by an investigation of storage proteins (Jakobashvili 1989). The stability of the *T. timopheevii* genome is possibly caused by the fact that all of the accessions, irrespective of their place of maintenance, were collected in a restricted region of Georgia.

T. militinae was found to be similar to *T. timopheevii* in C-banding pattern (Figs. 1, 2). Differences did not exceed the intraspecific variability of *T. timopheevii*. A similar storage protein composition has also been found for these species (Jakobashvili 1989). Thus, our data support the hypothesis that *T. militinae* occurred as a

Table 1 List of studied accessions (*AD* Amphidiploid, -accession number unknown)

Accession	Species	Identification of accession	Place of maintenance
1	*Triticum timopheevii*	–	Institute of Cytology and Genetics, Novosibirsk, Russia
2	*Triticum timopheevii*	Ii-380194	Vavilov Institute of Plant Industry, St. Petersburg, Russia
3	*Triticum timopheevii*	K-62644	Vavilov Institute of Plant Industry, St. Petersburg, Russia
4	*Triticum timopheevii*	–	Institute of Experimental Biology, Tallinn, Estonia
5	*Triticum timopheevii*	No. 21	Tbilisi Agricultural Institute, Tbilisi, Georgia
6	*Triticum timopheevii*	No. 23	Tbilisi Agricultural Institute, Tbilisi, Georgia
7	*Triticum timopheevii*	–	Institute of Botany, Georgia
8	*Triticum timopheevii*	ATRi 3433/SKL	Zentralinstitut für Genetik und Kulturpflanzenforschung, Gatersleben, FRG
9	*Triticum timopheevii*	TA 103	Kansas State University, Manhattan, USA
10	*Triticum militinae*	K-46007	Vavilov Institute of Plant Industry, St. Petersburg, Russia
11	*Triticum militinae*	Ii-084889	Vavilov Institute of Plant Industry, St. Petersburg, Russia
12	*Triticum zhukovskyi*	K-43063	Vavilov Institute of Plant Industry, St. Petersburg, Russia
13	*Triticum kiharae*	K-47897	Vavilov Institute of Plant Industry, St. Petersburg, Russia
14	*Triticum miguschovae*	K-57631	Vavilov Institute of Plant Industry, St. Petersburg, Russia
15	AD *T. timopheevii* × *T. tauschii*	TA 3432	Kansas State University, Manhattan, USA
16	*Triticum fungicidum*	K-40575	Vavilov Institute of Plant Industry, St. Petersburg, Russia
17	*Triticum timonovum*	K-43065	Vavilov Institute of Plant Industry, St. Petersburg, Russia
18	*Triticum timonovum*	No. 59	Tbilisi Agricultural Institute, Tbilisi, Georgia

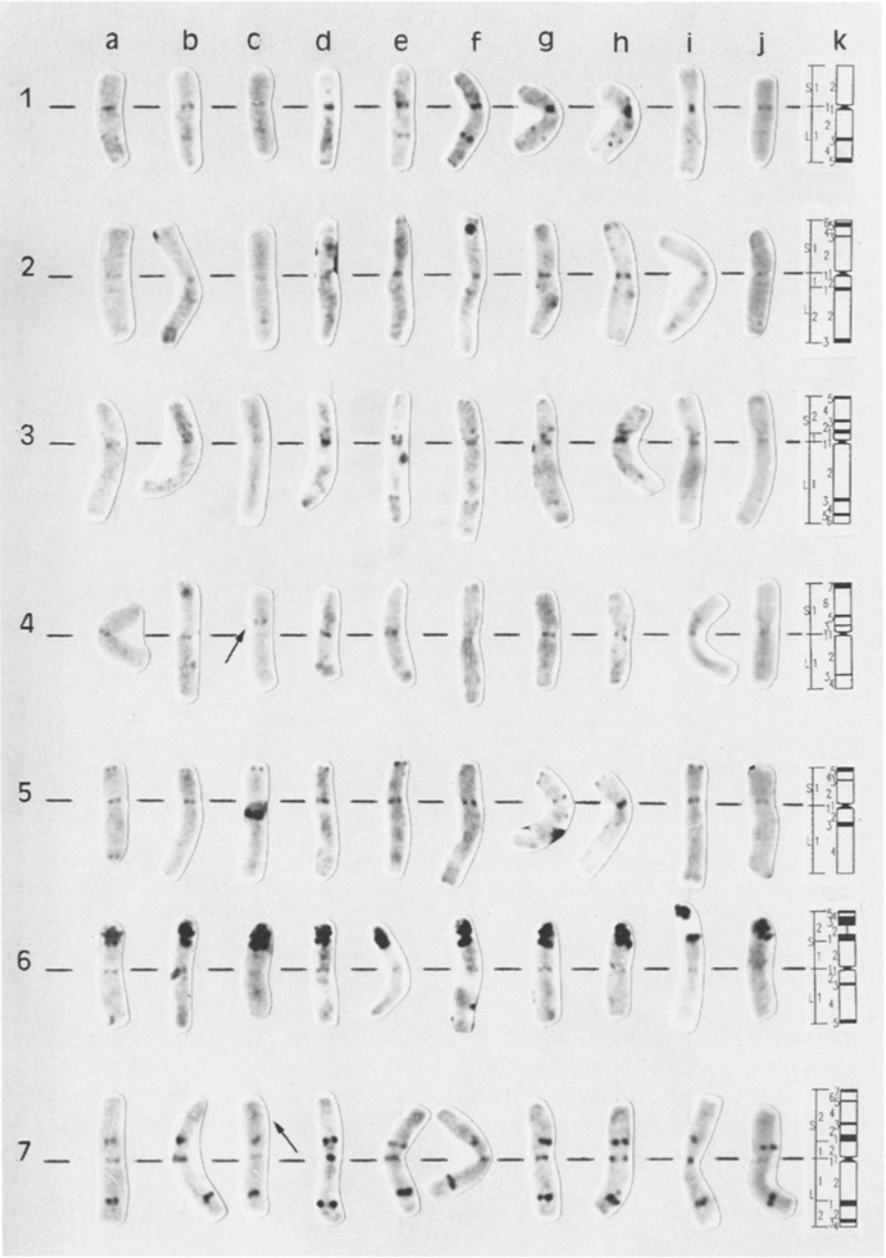

Fig. 1 C-banding patterns of the A^t-genome chromosomes in different lines of *Triticum timopheevii* (*a-i*) and *T. militinae* (*j*). *a* No. 1, *b* no. 8, *c* no. 9, *d* no. 2, *e* no. 3, *f* no. 5, *j* no. 10 (*T. militinae*)

result of gene mutation in *T. timopheevii* (Dorofeev et al. 1979).

A^t- and G-genome polymorphism in hexa- and octoploid species (*T. zhukovskyi*, *T. kiharae*, *T. miguschovae*, AD *T. timopheevii* × *T. tauschii*, *T. timonovum*, *T. fungicidum*)

The investigation of a *T. zhukovskyi* population showed that this species is stable with respect to both chromosome number and C-banding pattern. At the same time, 1 plant in the study was trisomic for one of the A^m-genome chromosomes, probably $2A^m$ (Fig. 3, trisomic chromosomes are denoted by arrows). A comparison of the C-banded karyotypes of *T. zhukovskyi* and *T. timopheevii* reveals a similarity of homologous chromosomes. At the same time, A^m-genome chromosomes, which were inherited from *T. monococcum*, were observed to be different from the A^t-genome chromosome of *T. timopheevii* in C-banding pattern, but similar to the chromosomes of the parental species, *T. monococcum* var 'hohensteinii' Flaksb. (described by Kuzmenko et al. 1987). It should be noted that some A^m- and A^t-genome chromosomes had similar C-banding patterns, i.e., the homoeologous group-7 chromosomes of both genomes

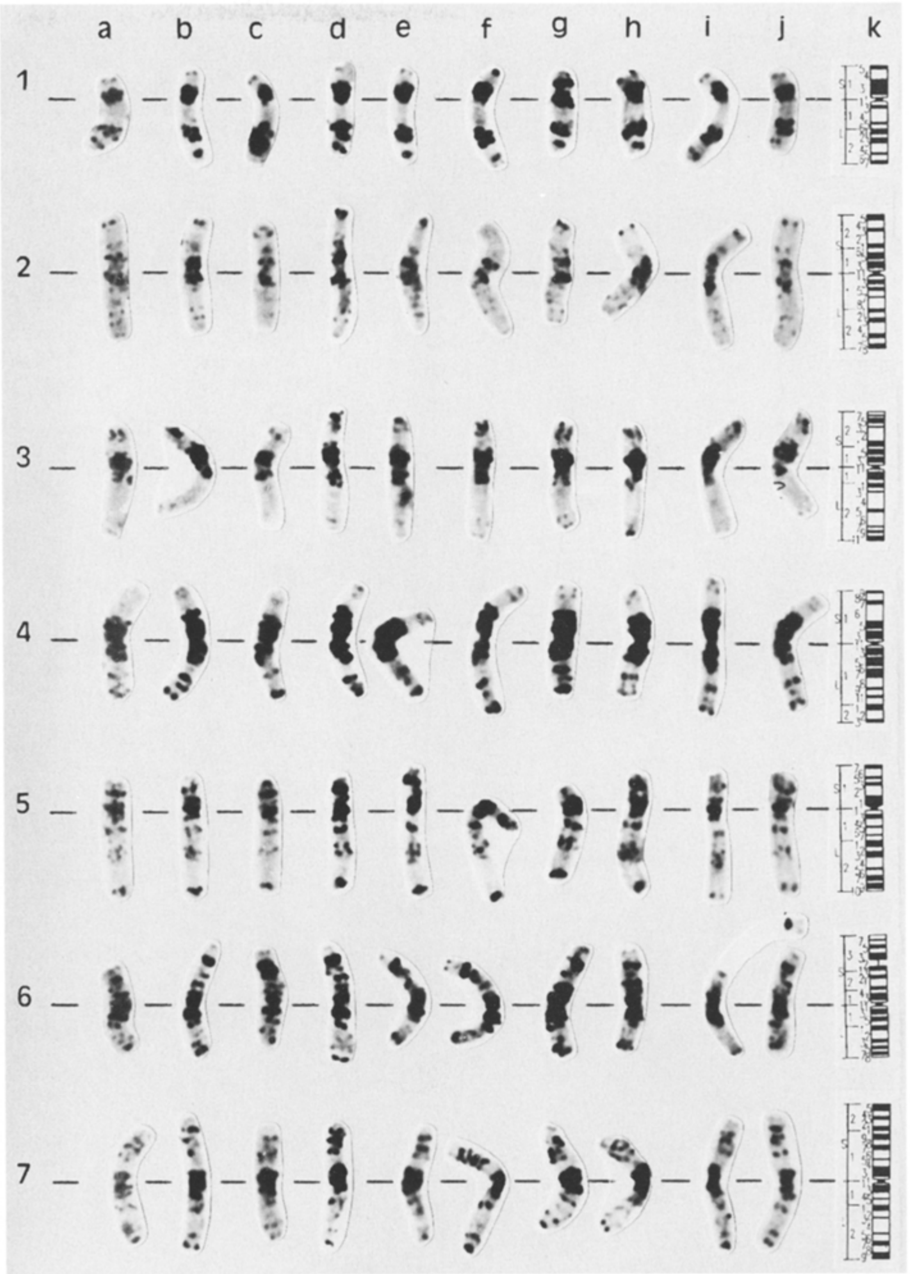

Fig. 2 C-banding patterns of the G-genome chromosomes in the karyotypes of different lines of *T. timopheevii* and *T. militinae*. The order of lines is the same as in Fig. 1

contained C-bands in the same position (Fig. 3), but in chromosome $7A^m$ polymorphism (presence/absence) of one of the intercalary C-bands was observed. We found a similar morphology and distribution of C-bands for the 5A chromosomes, although chromosome $5A^m$ has a more prominent telomeric band in the short arm. The $3A^tS$ and $4A^mS$ chromosome arms have a similar distribution of C-bands, but differ in the long arms. In general, this observation supports the hypothesis that a possible translocation occurred between the 3A and 4A chromosomes during speciation of *T. timopheevii* (Gill and Chen 1987).

T. kiharae, *T. miguschovae* and AD *T. timopheevii* × *T. tauschii* are the species with the same genome composition, A^tGD, derived from different cross combinations. Analysis of these species revealed a high similarity of their A^t- and G-genome chromosomes and differences in the D-genome chromosomes with respect to C-banding pattern (Fig. 4). In *T. kiharae* and AD *T. timopheevii* × *T. tauschii* the D-genome chromosomes had similar C-banding patterns, which differed from those in *T. miguschovae*. In this species, the D genome is similar with the genome of the parental *T. tauschii* line (Badaeva et al. 1989).

Thus, the A^t- and G-genome chromosomes in the karyotypes of 4 hexaploid species with different genome compositions were similar in C-banding patterns, both with each other and with the parental species. Since *T.*

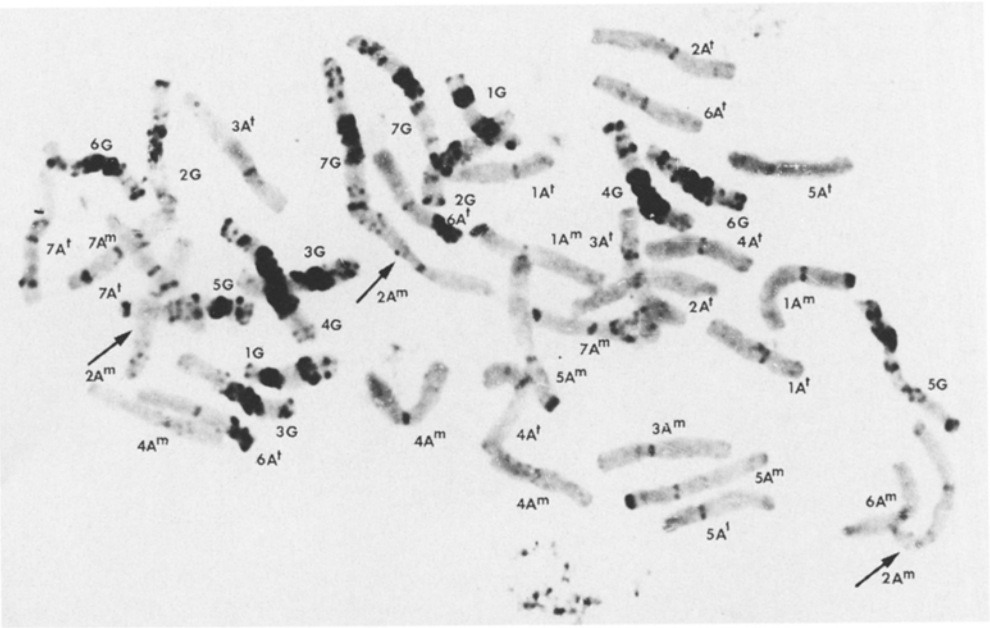

Fig. 3 A C-banded mitotic metaphase plate of *T. zhukovskyi* (no. 12), trisomic for the 2A^m chromosome (*arrows*)

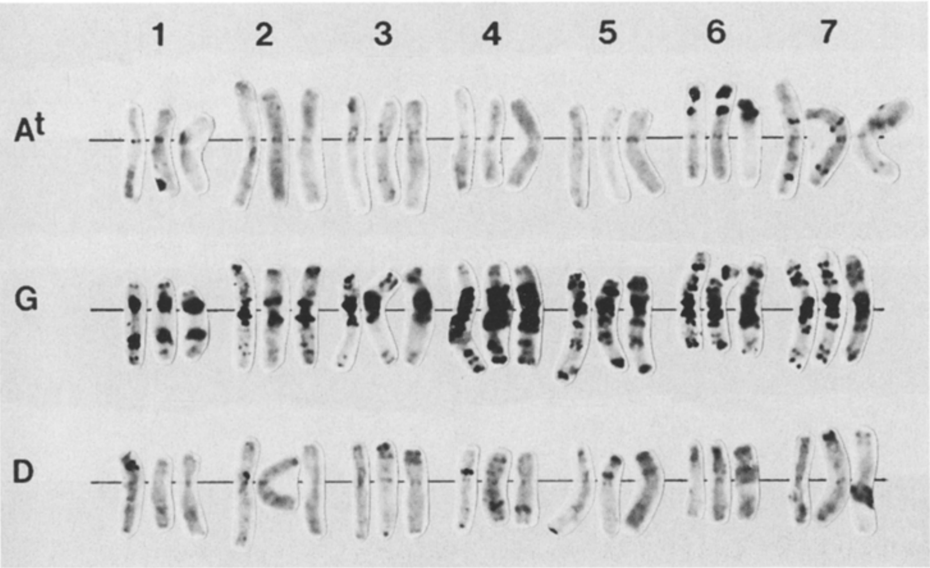

Fig. 4 C-banding patterns of the A^t, G- and D-genome chromosomes in *T. miguschovae* no. 14 (*left*), *T. kiharae* no. 13 (*middle*) and AD *T. timopheevii* × *T. tauschii* no. 15 (*right*)

zhukovski arose as a result of spontaneous hybridization and all allopolyploids with A^{t}GD genome composition are artificially synthesized, one may infer that differences in their origin had no effect on the function of parental genomes in their karyotypes. The same phenomenon was found with the spectrum of storage proteins (Jakobashvili 1989).

In general, the two studied forms of *T. timonovum* had a similar genome composition (Fig. 5), C-banding patterns and storage protein profiles. The karyotype of this species includes one set of *T. timopheevii* chromosomes, the B genome and an A^t genome with partial substitutions of A-genome chromosomes from an unidentified species of emmer wheat. Differences between the lines consist of a combination of A-genome chromosomes in homoeologous groups 1, 2, and 5.

The A, B, A^t and G genomes in the karyotype of *T. fungicidum* (Fig. 6) were similar to the parental species, *T. timopheevii* and *T. carthlicum* in their C-banding patterns (Badaeva et al. 1989). At the same time, the A and B genomes in this species were different from the corresponding genomes of *T. timonovum*. These data suggest that different species donated the A and B genomes to *T. fungicidum* and *T. timonovum*.

Comparison of A^t- and G-genome chromosomes in octoploid species shows similar C-banding patterns, both with respect to each other and with the parental species, *T. timopheevii*. At the same

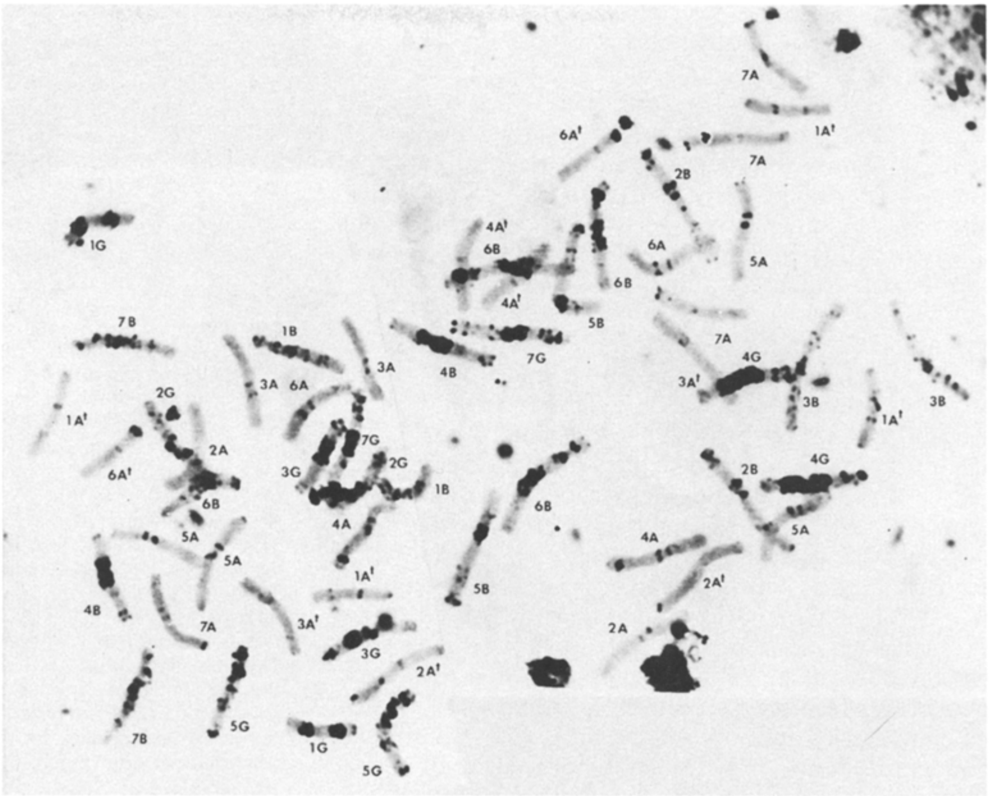

Fig. 5 A C-banded mitotic metaphase plate of *T. timonovum* (2n = 8x = 56) line no. 18

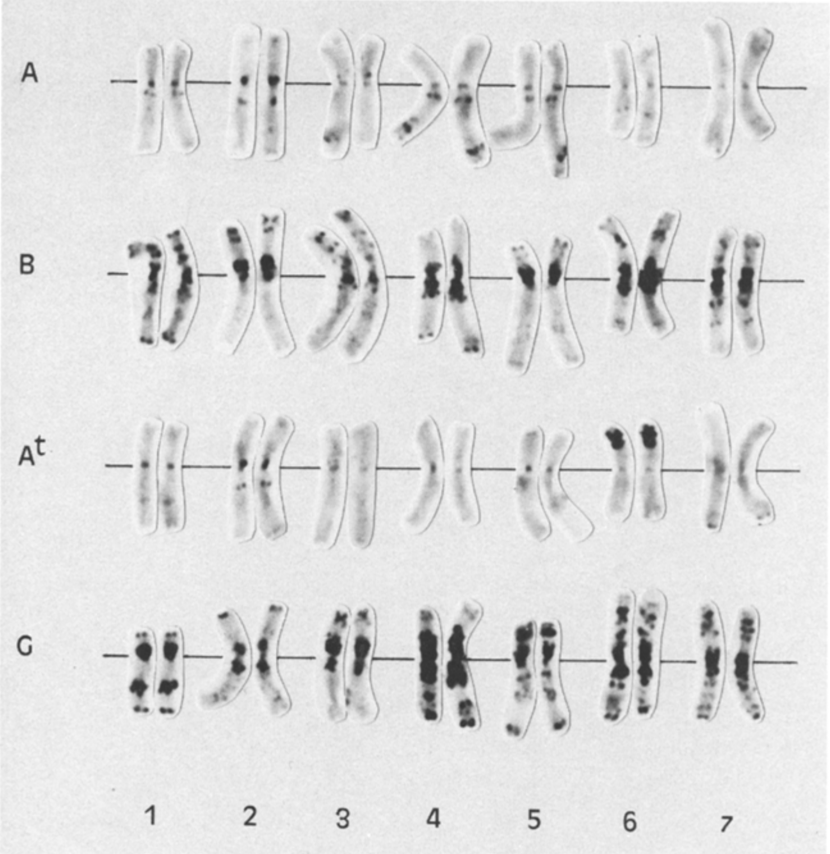

Fig. 6 The C-banded karyotype of *T. fungicidum* line no. 16 (2n = 56, ABA^{t}G)

time, octoploid species were characterized by a specific distribution of C-bands in chromosomes $2A^t$ and $7A^t$ (Figs. 5, 6).

The investigation of chromosomes of the A^t and G genomes of *T. timopheevii* and derivative species revealed a significant similarity in C-banding patterns irrespective of genome composition and ploidy level. These species either have a very narrow distribution in nature (like *T. timopheevii* and *T. zhukovskyi*), or were synthesized artificially and maintained in collections and nurseries (other species). Thus, the high conservation of the C-banding patterns in these forms is caused by the fact that they have not been under the strong pressure of natural selection at different environmental conditions. Another, possibly the most important, cause of high karyotypic stability of the *T. timopheevii* genome will be the short historical length of its existence (K. Tsunewaki, personal communication). The naturally growing wild species *T. araraticum*, with its comparatively wide area of distribution, was characterized by a high level of C-banding polymorphism. The second factor that may influence the decrease of polymorphism was the domestication of *T. timopheevii* in a restricted area of W. Georgia. Moreover, *T. timopheevii* as an endemic species with a restricted area of distribution is characterized not only by monomorphism of morphological features but also by a low variability of the C-banding patterns of the A^t and G-genome chromosomes.

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