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Cytogenetics of *Tradescantia spathacea* (syn. *Rhoeo spathacea*). A review

Cytogenetyka *Tradescantia spathacea* (syn. *Rhoeo spathacea*). Artykuł przeglądowy

SUMMARY

As a favorable PTH (permanent translocation heterozygosity) model, *Tradescantia spathacea* (synon. *Rhoeo spathacea*) offers an unusual opportunity to address crucial cytogenetic problems, among them complex chromosome rearrangements, recognition and segregation of translocated genome parts, the relation of crossing-over to synaptic processes. Here I present the existing knowledge on *T. spathacea* with the intention to reestablish it for advanced cytogenetic research.

Keywords: chromosomes, karyotype evolution, meiosis, meiotic ring, *Rhoeo*, permanent translocation heterozygosity (PTH), rDNA, *Tradescantia spathacea*, translocations

STRESZCZENIE

Tradescantia spathacea (syn. *Rhoeo spathacea*) jako tzw. permanentna heterozygota translokacyjna jest cennym organizmem modelowym do rozwiązywania ważnych problemów współczesnej cytogenetyki. Wśród nich są: kompleksowe chromosomowe rearanżacje, koniugacja i segregacja translokacyjnych segmentów, relacja pomiędzy *crossing-over* a procesami związanymi z koniugacją. W niniejszym artykule został przedstawiony istniejący stan wiedzy dotyczącej cytogenetyki *T. spathacea* oraz zostały poruszone najważniejsze zagadnienia z nią związane.

Słowa kluczowe: chromosomy, ewolucja kariotypu, mejoza, pierścień mejotyczny, *Rhoeo*, permanentna heterozygotyczność translokacyjna (PTH), rDNA, *Tradescantia spathacea*, translokacje

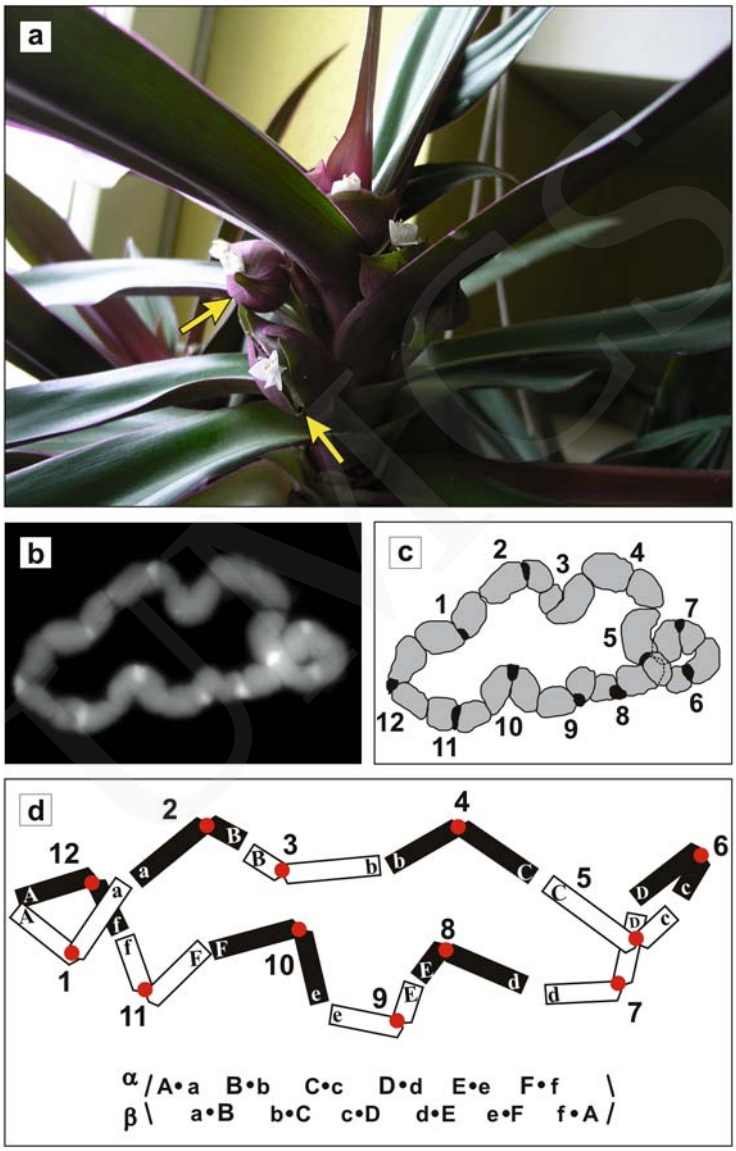

Fig. 1. *T. spathacea* (boat lily) grown in the pot as a houseplant – general plant morphology (1a) and chromosome arrangement within meiotic ring (1b, 1c, 1d). 1a – Purple-leaved common variety (var. spathacea) with upper leaf surface uniformly green and purple bottom surface; yellow arrows indicate boat-like bracts (spathae), i.e., modified and specialized floral leaves forming a tight sheath around inflorescences. Single white flowers can emerge like men from a boat. 1b – A metaphase I (MI) ring of twelve chromosomes joined by terminal chiasmata. Squash meiotic preparation stained with DAPI (4'-6-diamidino-2-phenyloindole) and photographed under epifluorescence microscope. The zig-zag (alternate) disjunctional arrangement is disturbed by squashing. AT-rich

pericentromeric heterochromatin fluoresces more intensively than the rest of chromosome portions. Two chromosomes (chromosomes 3 and 4) are unique, as they are poor with this genome fraction. 1c – graphical interpretation of Fig. 1b. The sequence of chromosomal types (1–12) is stable (species-specific). AT-rich centromeric heterochromatin drawn in black. 1d – a scheme illustrating regular alternate arrangement of kinetochores (red dots) within MI meiotic ring. Chromosomes belonging to *a* complex are white, while those of *β* complex are drawn in black. Homologous end-segments are labeled with letters, according to Belling's segmental interchange hypothesis. A letter-based chromosome formula of the ring was shown below. Note that heterobrachial chromosomes conjoin by their shorter arms.

INTRODUCTION

Tradescantia spathacea (Sw.) Stearn (Fig. 1a) appears a highly interesting diploid organism as a model for genome-wide complex chromosome rearrangements. As a result of multiple reciprocal translocations, a large meiotic ring of twelve chromosomes is formed (Figs. 1b, 1c) during meiosis (4, 15, 28, 33, 36, 53, 60, 62, 64, 81). In *T. spathacea*, there are no two fully homologous chromosomes in the karyotype. Instead, every chromosome is partially homologous to its two neighbors in the ring (Fig. 1d). The complete meiotic ring is preserved in populations by acquisition of a special genetic condition, the so-called permanent translocation heterozygosity (PTH). The growing interest on PTH organisms has recently resulted in applying techniques of both molecular cytogenetics and molecular genetics (33, 36, 76, 77) to bring a light on complex genome rearrangements. Although PTH is also found in *Oenothera* (12) and in a few other plant taxa (43), *T. spathacea* provides more favorable material. It has a low chromosome number (2n=2x=12) associated with relatively large chromosomes. Importantly, it is easy to cultivate, grows robustly and flowers during the whole year under optimal conditions. The aim of this review is presenting the existing knowledge on *T. spathacea* with the intention to reestablish its cytogenetics for the scientific community.

TAXONOMY, GENERAL CHARACTERISTICS

Tradescantia spathacea Sw. belongs to a monotypic section *Rhoeo* (Hance) Hunt, which is one of the twelve sections of the genus *Tradescantia* (family *Commelinaceae*, subfamily: *Commelinoideae*, tribe *Tradescantieae*), the latter contaning approximately 70 species (21, 46). Creating a separate *Rhoeo* section for *T. spathacea* appears substantiated by recent molecular approach, which demonstrated this species as separate clade (9). Before uniting with *Tradescantia* (46), the species was described as *Rhoeo discolor* (L'Herit.) Hance or *Rhoeo spathacea* (Sw.) Stearn, and existed within a separate monotypic genus *Rhoeo* Hance of the *Commelinaceae* family. The home of this plant is Mexico, Central America, Ca-

ribbean, West Indies, however by now it has been naturalized over a wide area outside its original range, mainly in temperate Asia, United States (Florida), Seychelles, Micronesia (58, 85). In Florida, it is regarded as an invasive plant, as it can escape cultivation and quickly expand ground cover, which may cause ecosystem problems (58). The plant has a short rosette-like stature (Fig. 1a), adapts to many different kinds of soils, grows robustly and flowers during the whole year under optimal conditions. Because the boat-shaped inflorescence bracts enclose slightly emergent white flowers (Fig. 1a), the popular names of this ornamental weed are: "boat lily", "Moses in a boat", "Moses in a cradle", "three men in a boat". *T. spathacea* is cultivated world-wide as a greenhouse or houseplant for its color and texture attributes. Apart from its ornamental value, it has been in use in traditional medicine (79). A common ring-forming variety (var. spathacea) possesses thick, sword-like leaves with the green upper surfaces and the undersides being shades of red or purple (Fig. 1a). There are also ring-forming variegated cultivars, described as *T. spathacea* var. variegata, with yellow and/or pink longitudinal stripes along the otherwise green upper leaf surface (2). Additionally, a rare anthocyjaninless variety – *T. spathacea* var. concolor Baker from Belize was described, possessing leaves totally green and growing sympatrically with some populations of the spathacea variety (97). The concolor phenotype likely segregates from the spathacea variety, but is not linked to any type of MI configuration. Some concolor clones may display the presence of the ring of twelve at meiosis (2, 60), while others form six bivalents (29, 74, 97). Apart from diploid forms, spontaneous triploids and tetraploids of the spathacea variety were reported (24, 66, 96). They appear to be due to occasional cleavage division errors and/or production of diploid gametes (24).

PERMANENT TRANSLOCATION HETEROZYGOSITY (PTH)

Permanent translocation heterozygosity (PTH) has developed independently in several plant families, e.g. *Onagraceae* (12), *Commelinaceae* (54, 80)*, Lobeliaceae* (49)*, Iridaceae* (37)*, Papaveraceae* (91). It has been widely assumed that during evolution towards PTH, reciprocal translocations have led to meiotic catenation, that is, to rings and/or chains at meiosis (12, 16, 64, 76). In a PTH organism there are two multichromosomal superlinkage groups, the so-called Renner complexes – α and β complex. Every second chromosome in the ring belongs to the same Renner complex and all the chromosomes of a given complex segregate as an unit because at metaphase I kinetochores are alternately orientated, which allows every second chromosome to go to the same pole (12, 73). Thus, In *T. spathaecea*, the co-segregating alternate chromosomes within a ring are genetically linked, forming an egg or a sperm translocation complex, each consisting of six chromosomes (Fig. 1d). The perpetuation of the heterozygous condition in a

PTH system, is accomplished by autogamy combined with a mechanism which eliminates homozygotic (α·α or β·β) progeny (12, 40, 43, 80, 92, 97).

In *Oenothera*, within a given chromosome of a PTH species, a series of ancient translocations most probably has generated a highly complex assemblage of differential segments, sharing partial homology with multiple regions on other (nonhomologous) chromosomes. Therefore, to avoid sterility (complicated missegregation figures leading to aneuploidy), the region perturbed by translocations was sheltered from ectopic recombination by damping down the homologous recombination (40). The shutdown of both the homologous recombination (HR) and free chromosome segregation, together with genetically uniform (clonal) and permanently heterozygous (α·β) population structure, are the reasons why PTH-breeding scheme is viewed as a paradoxical type of a "functionally apomictic" sexual reproduction (7, 16, 19, 43, 51, 52). Other similar to PTH situations, frequent in animal and plant taxa, including different degrees of translocation polymorphism or sex-linked translocation heterozygosity (8, 39, 78) differ from PTH with regard to the above mentioned features. PTH in plants has probably evolved as the ultimate way to maintain heterozy in face of inbreeding, and exists in those rare taxa who had been able to incorporate extensive reciprocal translocations as a stable part of their genetic system (43).

The unusual meiotic structure of *T. spathacea* – translocation ring of twelve was for the first time observed by Belling (4), who coined the idea of the segmental interchanges between non-homologous chromosomes as a mechanism generating multichromosomal rings in plants. Afterwards, Sax (81) reported a stable sequence of eight chromosomes of the ring and gave the first reliable information on their morphology. He divided the meiotic chromosomal types into two groups: isobrachials (i) and heterobrachials (h), and found that heterobrachial chromosomes conjoin by their shorter arms (Figs. 1b, 1c, 1d). Furthermore, he proposed letters designating all the homologous chromosome ends in the ring (Fig. 1d). The observations on standard morphology of meiotic chromosomes were then extended by Lin and Paddock (64), Lin (62), Golczyk et al. (33, 36), Golczyk (28). As a result, the coded sequence of twelve meiotic chromosomes: 1Aa(i) – 2aB(h) – 3Bb(h) – 4bC(i) – 5Cc(h) – 6cD(h) – 7Dd(i) – 8dE(h) – 9Ee(h) – 10eF(i) – 11Ff(i) – 12fA(i), was shown to be stable and invariably the same both in the common (spathacea) and in the variegated variety (variegata) of *T. spathacea* (Fig. 1d). The letter designations of end-segments are of course arbitrary, but they do show the homology relationships of different ends of the 12 chromosomes. It was assumed (23, 28, 33, 36, 64) that the α Renner complex consists of chromosomes 1(Aa), 3(Bb), 5(Cc), 7(Dd), 9(Ee), 11(fF), and the β complex encompasses the six remaining chromosomes: 2(aB), 4(bC), 6(cD), 8(dE), 10(eF), 12(fA). It is necessary that at least 6 of the 12 chromosomes have been produced by segmental interchange, but more likely is the *Oenothera* scenario, where most or all

the members of a karyotype are segmental interchange chromosomes (12). In *T. spathacea*, the ring of twelve is formed both in male (4, 15, 28, 33, 36, 53, 60, 62, 64, 81) and female (10, 92) meiosis. The plant is partially fertile, as in standard conditions 80–90 percent of the pollen grains can be morphologically imperfect (81) and about half of the chalazal megaspores eventually abort during the following divisions (10, 92). Most of these sterilities are the result of non-disjunction at anaphase I (1, 10, 92).

In *Oenothera*, PTH with some minor exceptions is based on frequent autogamy, however at the same time some low level of outbreeding is needed for generating new stable PTH hybrids/forms (microspecies). The properties of PTH have allowed, through infrequent intra- and interspecies hybridization events between distinct translocation lineages, the immediate and permanent fixation of new sets of features which ensured the success in colonizing new environments *via* creating new *Oenothera* PTH microspecies (19). In *Oenothera*, a complete PTH meiotic ring can be formed as a result of one hybridization event between two lineages differing by chromosome configurations at metaphase I (12). Unfortunately, *Rhoeo* section is a monotypic clade and no data exists on the role of relevant hybridization events. Bearing in mind the apparent uniformity of *Rhoeo* (=*T. spathacea*) and the lack of translocation polymorphism, this taxon may represent a blind evolutionary alley (86). The studies of Carniel (10) and Tschermak-Woess (92) indicate that in *T. spathacea* the chalazal cell of the megaspore tetrad always gives rise to the embryo sac. Thus, the so-called Renner effect, i.e, gametophyte competition, which is frequent in *Oenothera*, does not seem to be operating. Since megaspore or megagametophyte elimination does not occur, zygotic or embryonic lethality must be operating (100). It seems unlikely that lethals are due to sectional deficiencies in one or both complexes, because bivalent-forming derivatives of the spathacea variety, known as concolor variety, may contain one of the two complexes in double dose (29, 74, 97). Golczyk (29) has recently shown that the bivalent-forming *R. spathacea* var. concolor consists of two β complexes (Fig. 2b). Hypothetically, l_1 and l_2 zygotic lethals may participate in establishing the fertile balanced heterozygotic genotype L_1l_2/l_1L_2 in a repulsion phase linkage, as it is supposed to be the case in *Oenothera lamarckiana* (38). If balanced lethals in the ring-forming *Rhoeo* are situated within conjugating homologous chromosome arms, sporadic recombination events between the two otherwise non-recombining Renner complexes in the region where the lethals are located, may potentially release a lethal recessive from one of the complexes by a simple transition from L_1l_2/l_1L_2 to L_1L_2/l_1l_2, thus yielding a homozygotic L_1L_2/L_1L_2 progeny (29). In *Oenothera lamarckiana*, the release of a zygotic lethal is conditioned by a series of segmental rearrangements between the two, *gaudens* and *velans* Renner complexes, as a result of which an alethal complex, which is a mixture of *gaudens* and *velans* is formed, and after selfing can

be realized in a homozygotic condition (12, 20, 40). In general, in *Oenothera*, vigorously growing homozygous strains, let us say – "translocation homozygotes" can segregate on different occasions from PTH forms (l.c.). Thus, there are potent switches from functionally apomictic non-Mendelian PTH route (ring of fourteen) to standard Mendelian mode of inheritance (7 bivalents). An analogous situation is likely to be the case in the PTH system of *T. spathacea*.

MEIOTIC PAIRING, CHIASMATA, CROSSING-OVER

The genetic condition of the PTH system reflects a highly stringent compromise between two seemingly conflicting evolutionary forces – the wholesale damping down of recombination and a need for a regular (due to chiasmata) segregation at meiosis. While most portion of Renner complexes, i.e., their non-recombining part stays essentially uncontaminated through generations, obligatory HR-events between pairing chromosomal ends accomplish the task of assembling terminal chiasmata, which are crucial for regular alternate disjunction (12, 40, 59, 86). The pairing- and recombination-patterns prior to MI, influence strongly disjunction irregularities which may account for 40%–50%, or even 80% pollen sterility (1, 15, 57, 81, 82, 95). Spindle interactions at prometaphase/MI, present themselves another source of pollen decay. Together with disjunctional aberrations and environmental factors they can cause pollen abortion in *T. spathacea* rise to 80–90%, or even to 100% (95, 100).

The available data indicates that the terminal chiasmata formation in *T. spathacea* is likely to be under genetic control (24, 25, 75, 94). Interstitial chiasmata in *T. spathacea* were reported only sporadically (18, 23, 94, 96). An extensive relaxation of the genetic control of chiasma pattern, not linked to horticultural conditions, was reported only once by Verma and Ohri (94). In this study complex configurations resulted from pairing not limited to the end-segments, but extended to the interstitial and the differential segments (cf. 16), were indicated. Remarkably, this complete breakdown of the meiotic system generated curious smaller rings so far not found by other researchers, and resulted in total pollen sterility. What mechanism stands exactly behind the terminal positioning of the crossovers, remains one of the main cytogenetic problems to resolve (44). It can not be excluded, that the highly specialized PTH system involves some special mechanism, unique to PTH itself. Interestingly, Williams and Heslop-Harrison (98) have observed unusual peripheral membranous structures associated with the *Rhoeo spathacea* meiotic chromosomes. These structures have no obvious affinity to any other previously described chromosome-associated bodies, and, as was stated by the authors, they may form bridges between the ends of the adjoining chromosomes. It was shown that both synaptic failure and chiasma failure have the same frequency and occur randomly among the 12 pairs of chromosomal ends

(60, 65, 84). The conclusion was that crossing-over is likely confined to small terminal segments and the cause of chain formation is the failure of synapsis (l.c.). However, the strength with which chiasma failure proceeds, can be significantly modified by environmental factors, e.g. temperature (41, 63, 81). When chiasmata fail in one or more pairs of homologous ends, one or several chains can be formed instead of a complete ring. Not surprisingly, the scored frequencies of the complete ring are within quite a broad range, i.e., from ca. 2% (48) through diverse intermediate levels (68, 94, 100), up to 78% (84). Furthermore, if the conditions are far from optimal (e.g., very low temperature), complete asynapsis could be introduced (81). Regulatory mechanisms facilitating HR restriction may include alterations in timing and extent of both pairing and synapsis, chromatin structure, as well as molecular machinery affecting recombination (42, 47, 99). An easy explanation for dysfunctions in crossovers in a non-recombining system could be just a programmed failure to form synaptonemal complex (SC) within regions secured from recombination. In *T. spathacea*, whole-mount SC spreads indicate that telomere-led synapsis is never completed and is confined to distal chromosome segments of ca. 1/3 arm length (84). Thus, the extent of SC is substantially longer than could be expected, if only chromosome termini were pairing. Moreover, classical cytological observations conducted by Koller (56) and serial 3D EM reconstructions from sectioned meiotic nuclei (61, 70), strongly suggest that chromosomes of *T. spathacea* are synapsed throughout their substantial length. Synapsis-promoted juxtaposition of chromosome arms or their substantial parts could be important for a translocation system, as potentially facilitating alternate disjunction (78). Indeed, growing body of evidence indicates that SC may function as a dysjunctional device, promoting biorientation of the segregating chromosomes (27, 67, 78, 89). Moreover, a condition of chromosome regions being both synapsed and restricted from HR is not an odd curiosity, as synapsis can be uncoupled from HR (69). Besides, SC elements may be modified in such a way, as to cause a reduction in the formation of COs (22). Both in *Oenothera* and *T. spathacea*, telomeres group around zygophase (34, 71, 84). Notably, in both taxa there is an additional untypical nuclear constraint – Rabl arrangement generated by clustering of centromeric regions, observed before zygotene pairing and maintained for a prolonged time during meiotic prophase I (30, 34, 84). This contrasts with what is found in other organisms, where centromere associations are usually resolved prior to zygotene pairing (11, 50, 87). Such an additional meiotic constraint, may potentially promote homology search and pairing in face of SC- and HR- dysfunctions (3). Indeed, in *T. spathacea*, the role of chromocenters in pairing as well as in the determining the number of meiotic chains (1–3 collective chromocenters = 1–3 meiotic chains), has been already proposed (13, 72).

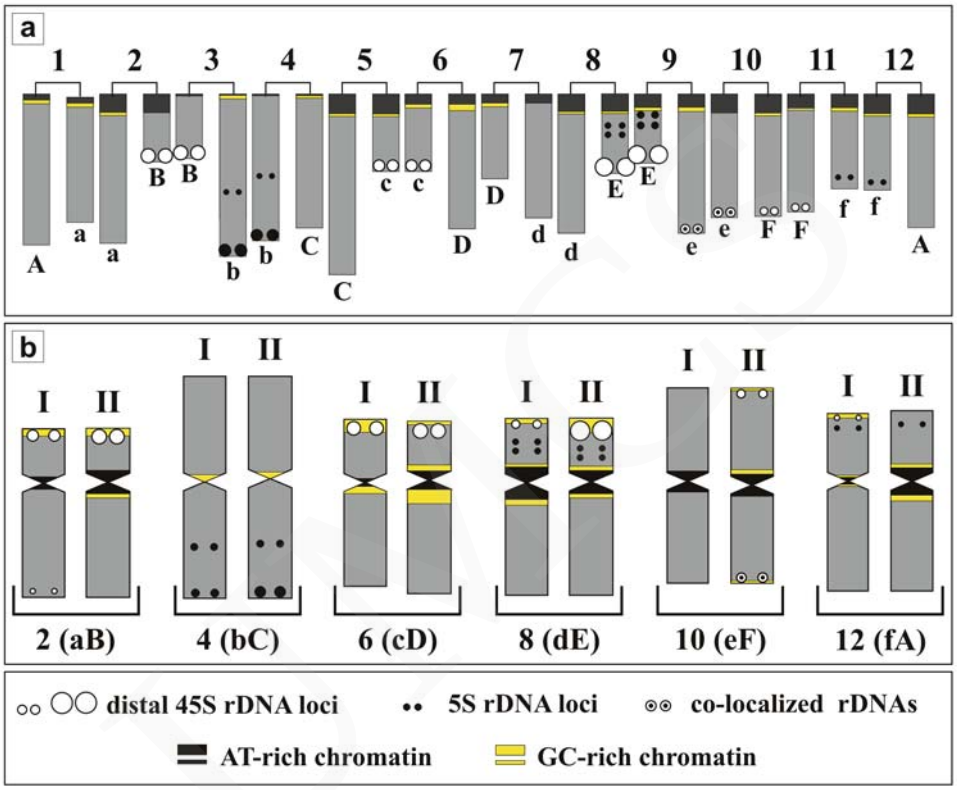

Fig. 2. Karyotype structure in *T. spathacea*. 2a – Karyotype of the two ring-forming varieties (vars. spathacea and variegata). Chromosome arms are bent to graphically depict length relations. There is no significant karyotypic difference between the two varieties. The measured chromosomes (1–12) arranged in the meiotic sequence. Chromosomes 2, 3, 5, 6, 8, 9 have 45S loci localized distally on their shorter arms. Chromosome 10 has two 45S rDNA loci each localized distally on one arm. Chromosome arms 9e and 10e have colocalized distal 5S and 45S rDNA loci. Each of the chromosomes 3 and 4 has distal 5S rDNA locus on its arm b. Interstitially located 5S rDNA loci are on chromosomes 3, 4, 8, 9. Small 5S rDNA loci on the shorter f arms of chromosomes 11 and 12 are also localized interstitially. The duplicated 5S rDNA locus (consisting of two smaller loci separated by a short distance) residing on each of 8E and 9E arms, may have originated *via* a small inversion with one of the breakpoints within the already existing 5S rDNA cluster. Each of the two interstitial 5S rDNA loci on 3b and 4b chromosome arms may have originated *via* a middle-sized segmental inversion with one of the breakpoints within the telomere-adjacent major 5S rDNA locus. In both ring-forming varieties, the total sum of length mismatches ranges around 6% of the genome (28, 36), thus it is close to the total amount of AT-rich pericentromeric heterochromatin (ca. 8%). This fact suggests that all the length mismatches are likely the result of translocation breakpoints occurring within or near AT-rich centromeric heterochromatin, but not exactly at the same distance from the centromeres. Thus, WATs seem a reasonable explanation as an important mechanism of genome-wide chromosome repatternings in the section *Rhoeo*. 2b – the alethal $^{h}\beta$ complex (**I**) of the bivalent-forming concolor variety is strikingly similiar to the β complex (**II**) of the ring-forming varieties, only slightly modified. The concolor variety has likely segregated from the ring-forming *T. spathacea*.

KARYOTYPE STRUCTURE AND EVOLUTION

As a result of applying an array of cytogenetic techniques, the chromosomal arrangements of rDNA sites (45S and 5S rDNA), NORs (nucleolus organizer regions), AT/GC-rich genome fractions were uncovered (28, 29, 32, 33, 35, 36) in each the three *T. spathacea* varieties (vars. *spathacea*, *variegata*, *concolor*) – see Fig. 2 for the details and further discussion on karyotype structure. All chromosomes but two (chromosomes 3 and 4) have large amount of constitutive heterochromatin, mostly AT-rich, around their centromeres (Figs. 1b, 1c, 2a). The base-specific fluorescence revealed that in each pericentromeric region the central AT-rich domain is accompanied by one or two GC-rich clusters. Interestingly, dispersed 45S rDNA colocalizing with pericentromeric CMA-bands, was detected within all the pericentromeres in all the three varieties (28, 29, 36). In all the three varieties, pericentromeric rDNA is transcriptionally inactive, and all the terminal 45S rDNA clusters are NORs (28, 29, 36).

The obtained karyotypic data has led to a proposal (28, 36) that during evolution of the PTH in *T. spathacea*, extensive rearrangements seem to have occurred in such a way as to preserve a high degree of length similarity between the arms that conjoin within the meiotic ring (Fig. 2a). The simplest way to achieve this are whole arm translocations (WATs) accompanied by paracentric inversions. It was proposed (l.c.) that subtelomeric 45S rDNA and pericentromeric chromatin may have served as putative breakpoint sites, generating whole-arm translocations and/or whole-arm inversions. Active 45S rDNA is frequently involved in rearrangements (6, 26, 83, 90, 93). Recently, it was shown that plant 45S rDNA loci are both replication- and transcription-dependent fragile sites mediated by epigenetic alterations associated with the failure of 45S rDNA to condense (45). The persistence of the cytologically well-defined chromatin domains (AT- and GC-rich chromatin, 45S rDNAs) within all the twelve pericentromeres indicates spreading and homogenization of their sequences during evolution of *Rhoeo* karyotype. A proposed scenario for testing is that once a subtelomeric DNA sequence has been transferred to pericentromeric region by translocation or whole-arm paracentric inversion, it could have spread over the non-homologous pericentromeric regions due to physical proximity of pericentromeres (collective chromocenters) during interphase and meiotic prophase. The latter process facilitates both WATs and spreading/homogenization of pericentromeric DNA sequences (36). Possibly, many ancient peracentric inversions of different size could have been involved in the evolution of the PTH karyotype in the section *Rhoeo* (see Fig. 2 for details). Paracentric inversion seems the only structural change which can efficiently restrict recombination, has no impact on chromosome morphology and is potentially able to cooperate with WATs in creating beneficial

linkages (55). Interestingly, the detection of interstitial telomeric $(TTTAGGG)_n$ DNA clusters in *T. spathacea* (14), may support the postulated scenario, that the regions protected from HR have been perturbed by ancient paracentric inversions. In diverse *Tradescantia* species and hybrids, inversions of different size frequently occur throughout the chromosomal length (5, 15, 17, 88). Recently, small subterminal inversions were implicated in the closely related *Tradescantia virginiana* (31).

CONCLUSIONS

As a favorable PTH model, *T. spathacea* gives us an occasion to resolve crucial cytogenetic problems, which are briefly addressed here. Among them are mechanisms of complex chromosome rearrangements, recognition and segregation of translocated genome parts, the relation of crossing-over to synaptic process. Combining molecular biology with cytogenetics should give us a closer look at the fascinating area of PTH system.

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