

Karyotype evolution in wasps of the genus *Trypoxylon* (subgenus *Trypargilum*) (Hymenoptera, Sphecidae)

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ABSTRACT

We report the karyotypes of five wasp species of the genus *Trypoxylon* (subgenus *Trypargilum*): *T. nitidum* ($2K = 10A^M + 18A + 2\overline{M}^{CC}$), *T. lactitarse* ($2K = 28A^{MC} + 2\overline{M}^{CC}$), *T. sp1* ($K = 3A^M + 10A + 2\overline{M}^{CC}$), *T. sp2* ($2K = 20A^{MC} + 12A$) and *T. sp3* ($2K = 2A^{MC} + 30A$). A comparison of the karyotypes of these species showed a small variation in chromosome number ($2n = 30$ and 32). However, much greater variation was observed when total arm number was compared ($2AN = 34$ to 60). This variability is mainly attributed to the difference in the amount of constitutive heterochromatin present in the karyotypes of these species.

INTRODUCTION

The genus *Trypoxylon* (Hymenoptera, Sphecidae) is divided into two subgenera: *Trypoxylon* and *Trypargilum*. The subgenus *Trypargilum* is restricted to the western part of the Southern hemisphere, with a greater diversity of species in the neotropical regions (Bohart and Menke, 1976; Coville, 1982; Amarante, 1991). All *Trypoxylon* species provision their nests with spiders. Some species construct mud nests while others use preexisting tubular cavities, which they divide into a linear series of cells with mud partitions. Like most Sphecidae, *Trypoxylon* wasps are solitary and the female constructs and supplies her own nest. In the subgenus *Trypargilum* the males mate with females during nest construction and provisioning, and guard the nest until it is completed (Coville, 1982).

At present, approximately 960 species of Hymenoptera belonging to 11 subfamilies have been analyzed cytogenetically (Imai, 1969; Crozier, 1975; Naito, 1978a,b, 1982; Imai *et al.*, 1984a,b, 1986, 1988a,b,

1994; Imai and Taylor, 1989; Pompolo and Takahashi, 1990; Hoshiba and Imai, 1993). In the genus *Trypoxylon*, only seven species have been studied at the cytogenetic level (Hoshiba and Imai, 1993; Gomes *et al.*, 1995).

For a better understanding of the mechanisms involved in the karyotype evolution of Hymenoptera and of *Trypoxylon* wasps in particular, a cytogenetic study on five wasp species belonging to this genus (subgenus *Trypargilum*) was conducted.

MATERIAL AND METHODS

The species were collected in Viçosa (MG) by the trap-nest technique (Krombein, 1967). These nests consist of blocks of perforated wood or bamboo of variable length and diameter in which the wasps construct their nests. The blocks were placed in different locations in the Viçosa region and were inspected periodically. After nest founding, the nests that had been completed were collected and taken to the laboratory where individuals in the prepupal phase

were selected for cytogenetic study. At least one individual per nest was allowed to emerge for later identification by a specialist. Samples of the species investigated are available in the Departamento de Biologia Geral, Universidade Federal de Viçosa.

The following species were studied: *Trypoxylon* (*Trypargilum*) *nitidum* F. Smith, 1856, *Trypoxylon* (*Trypargilum*) *lactitarse* Saussure, 1867 and three unidentified species, here designated *Trypoxylon* (*Trypargilum*) sp1, *Trypoxylon* (*Trypargilum*) sp2 and *Trypoxylon* (*Trypargilum*) sp3. Metaphases with good definition were obtained by the technique of Imai *et al.* (1988a), which has proved to be the most efficient for the Hymenoptera. C-banding was performed by the method of Sumner (1972), modified by Pompolo and Takahashi (1990). These procedures involve the following treatments: after the three days needed for preparation, the slides are 1) hydrolyzed by immersion in 0.2 N HCl for a variable period of time (3 to 5 min), 2) washed in distilled water for approximately 30 s, 3) immersed in 5% Ba(OH)₂ in a water bath at 60°C for a variable period of time (5 to 7 min), 4) washed in 0.2 N HCl for approximately 30 s and then in distilled water, 5) immersed in 2 x SSC solution (sodium citrate and sodium chloride saline), pH 7.0, in a water bath at 60°C for a variable period of time (6 to 8 min) and then in distilled water, 6) stained with Giemsa (Merck) in 8% 0.01 M phosphate buffer, pH 6.8, and carefully washed in running water.

Some C-bands were obtained from slides submitted to standard staining. After the slides were analyzed and photographed, they were slanted on filter paper to drain the immersion oil and then washed with a battery of three flasks containing xylene, 5 min in each flask. The slides were then dried, destained by immersion in 0.2 N HCl and submitted to the procedures described earlier for C-banding, with destaining corresponding to the first step in the procedure.

The slides were examined under the light microscope using an immersion objective. The metaphases of best quality were selected and photographed with a Zeiss photomicroscope, with an immersion objective using Agfa Copex Pan A.H.U. film. Chromosome morphology was described according to the classification used by Imai (1991) (Figure 1). For the description of chromosome and arm numbers we used the terms proposed by Imai and Crozier (1980) and by Imai *et al.* (1984a), whereby 2N and 2AN correspond to the diploid chromosome number and to the diploid arm number, respectively, and N and AN to the haploid numbers. The karyotypes were documented in the form of karyograms, with the chromosomes arranged in decreasing order of euchromatin regions.

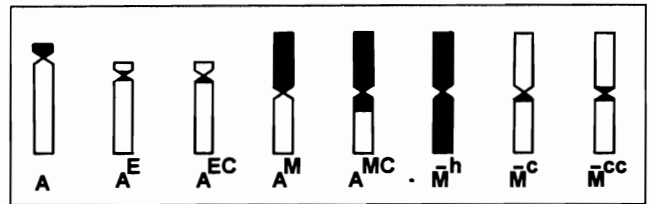


Figure 1 - Schematic presentation and nomenclature used for the types of chromosomes detected in the *Trypoxylon* species studied, according to the pattern of constitutive heterochromatin distribution (Imai, 1991). A, Acrocentric chromosome with a heterochromatic short arm; A^E, acrocentric chromosome with both arms being euchromatic; A^{EC}, acrocentric chromosome with heterochromatin in the proximal region of the centromere of the long arm; A^M, acrocentric chromosome (pseudoacrocentric) with a highly developed short arm due to growth of constitutive heterochromatin, often much longer than the euchromatic arm; A^{MC}, acrocentric chromosome (pseudoacrocentric) with a totally heterochromatic arm and with a heterochromatin block in the proximal region of the centromere of the euchromatic arm; M^h, fully heterochromatic meta- or submetacentric chromosome; M^C, meta- or submetacentric chromosome with a heterochromatin block in the proximal region of the centromere of one of the arms; M^{CC}, meta- or submetacentric chromosome with a heterochromatin block in the proximal region of the centromere of both arms.

RESULTS

The karyotype of *T. nitidum* ($n = 15$ and $2n = 30$) consists of 10 pseudoacrocentric A^M chromosomes (pairs 1, 6, 8, 10 and 15), 18 acrocentrics and two M^{CC} metacentrics (pair 14) (Figure 2A and B). The karyotype of the females of this species presents heteromorphism of pair 1, in which one of the chromosomes has a shorter long arm than the long arm of its homologue (Figures 2B and 7A). Analysis of the C-banding patterns showed heterochromatin throughout the extension of one of the arms in the pseudoacrocentric chromosomes, on the short arm, in the pericentromeric region of the acrocentrics, and in the pericentromeric region of metacentric chromosomes (Figures 2B and 7A). The diploid karyotype formula consists of $2K = 10A^M + 18A + 2M^{CC}$ and the diploid arm number is $2AN = 42$ (Table I).

The karyotypic constitution of *T. lactitarse* ($n = 15$ and $2n = 30$) is 28 pseudoacrocentric A^{MC} chromosomes and 2 M^{CC} metacentrics (pair 1) (Figure 3A and B). The entire extension of one of the arms, the proximal region of the centromere of the other arm of pseudoacrocentric A^{MC} chromosomes and the pericentromeric region of the metacentric M^{CC} pair were C-banding positive (Figure 3A and B and 7B). The diploid karyotype formula is $2K = 28A^{MC} + 2M^{CC}$ and the diploid arm number is $2AN = 60$ (Table I).

Some pairs heteromorphic with respect to the C-banding patterns seem to exist. The heterochromatin of the pericentromeric region of the euchromatic arms of some A^{MC} chromosomes was more abundant in one

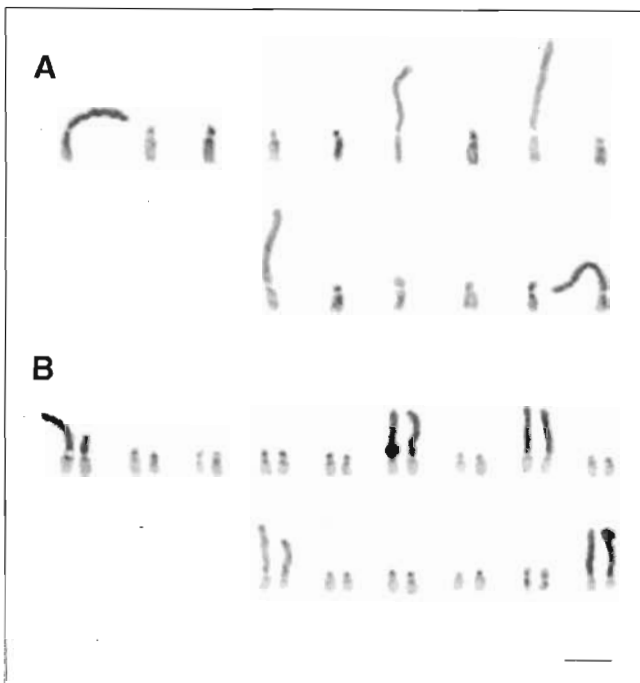


Figure 2 - *Trypoxylon (Trypargilum) nitidum*. A, Male karyotype ($n = 15$), standard staining; B, C-banding patterns in the karyotype of a female ($2n = 30$). Bar = 5 μm .

of the homologues of this species. Another polymorphism detected in this species involves the morphology of pair 15 (Figure 9). In some of the females analyzed this pair consists of two submetacentric chromosomes (SM/SM), a homomorphic pair, and in others it consists of a metacentric chromosome and a submetacentric chromosome (M/SM), a heteromorphic pair. No individual was detected in which the pair consisted of two metacentric chromosomes (M/M), although this was expected (Figure 7B). Among the males analyzed, both the metacentric chromosomes and the submetacentric chromosomes were detected (Figure 7B). This polymorphism was detected in individuals from the same nest and in individuals from different nests.

The haploid chromosome complement of *Trypoxylon* sp1 consists of 15 chromosomes (Figure 4).

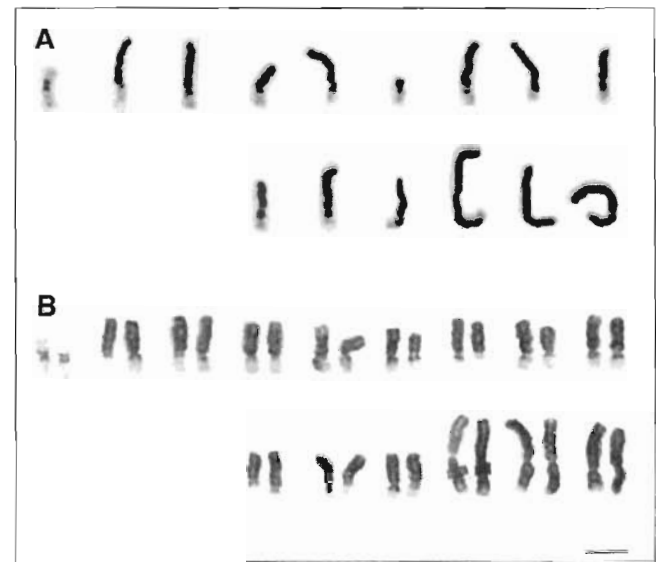


Figure 3 - *Trypoxylon (Trypargilum) lactitarse*. A, C-banding patterns of a male karyotype ($n = 15$); B, C-banding patterns of a female karyotype ($2n = 30$). Bar = 5 μm .

In the differential staining pattern observed in some metaphases three chromosomes (nos. 6, 10 and 12) presented a more deeply stained long arm and proximal region of the centromere of the short arm, indicating that they are heterochromatic, and were therefore called A^{MC} pseudoacrocentrics. Two chromosomes (nos. 1 and 14) were more deeply stained in the pericentromeric regions and were probably \overline{M}^{CC} , and the remaining ones were acrocentric chromosomes, a pattern suggesting that constitutive heterochromatin is located on the short arm and in the pericentromeric region (Figures 4 and 7C). The haploid karyotype formula is $K = 3A^M + 10A + 2\overline{M}^{CC}$ and the haploid arm number is $AN = 20$ (Table I).

The karyotype of *Trypoxylon* sp2 consists of 32 chromosomes. The karyotype of the females of this species presents heteromorphism in pair 14, similar to that detected in pair 1 of *T. nitidum* (Figure 5 and 8A). The differential staining pattern observed in some metaphases indicates that chromosomes 1, 2, 6, 8, 10, 12,

Table I - Collection sites, number of nests, of individuals and of chromosomes, karyotypic formula and arm number of five species of wasps of the genus *Trypoxylon* (subgenus *Trypargilum*) collected in the region of Viçosa, MG.

Species	No. of nests	No. of individuals		Chromosome number		Karyotype formula	Arm number
		M	F	n	2n		
<i>T. nitidum</i>	11	11	30	15	30	$2K = 10A^M + 18A + 2\overline{M}^{CC}$	$2AN = 42$
<i>T. lactitarse</i>	14	18	30	15	30	$2K = 28A^{MC} + 2\overline{M}^{CC}$	$2AN = 60$
<i>T. sp1</i>	1	2	-	15	-	$K = 3A^M + 10A + 2\overline{M}^{CC}$	$AN = 20$
<i>T. sp2</i>	2	-	9	-	32	$2K = 20A^{MC} + 12A$	$2AN = 52$
<i>T. sp3</i>	1	2	4	16	32	$2K = 2A^{MC} + 30A$	$2AN = 34$

AN = Arm number of the haploid karyotype; $2AN$ = arm number of the diploid karyotype; K = haploid karyotypic formula; $2K$ = diploid karyotypic formula.

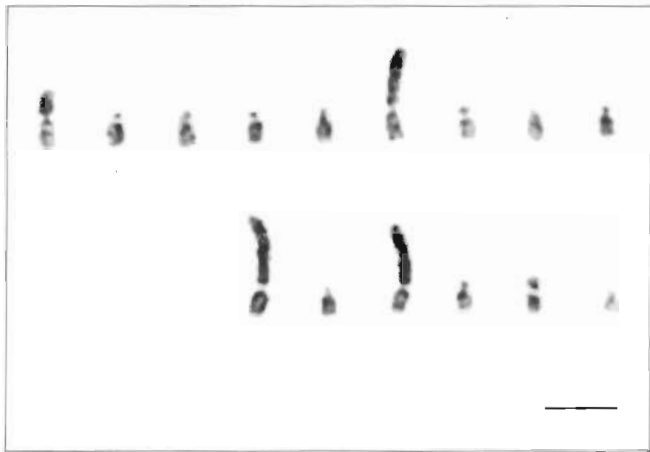


Figure 4 - *Trypoxylon (Trypargilum)* sp1. Male karyotype ($n = 15$) with standard staining. Bar = 5 μm .

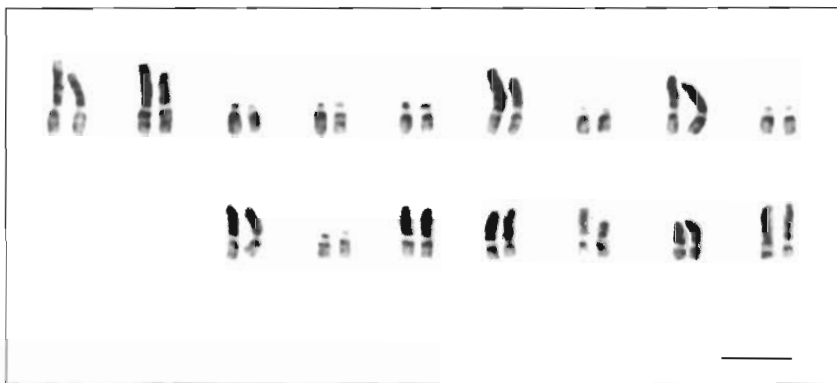


Figure 5 - *Trypoxylon (Trypargilum)* sp2. Female karyotype ($2n = 32$) with standard staining. Bar = 5 μm .

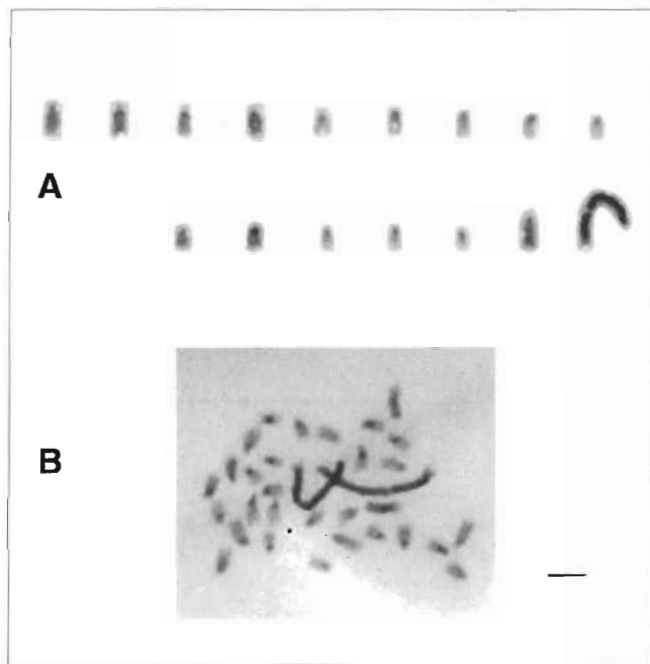


Figure 6 - *Trypoxylon (Trypargilum)* sp3. A, Male karyotype ($n = 16$) with standard staining; B, C-band in a female metaphase ($2n = 32$). Bar = 5 μm .

13, 14, 15 and 16 have darker long arms and a darker proximal region of the centromere of the short arms, and were therefore called pseudoacrocentric. The acrocentric chromosomes 3, 4, 7, 9 and 11 were more deeply stained on the short arm and in the pericentromeric region. This differential staining pattern suggests that the darker regions correspond to those of constitutive heterochromatin (Figures 5 and 8A). The diploid karyotype formula is $2K = 20A^{MC} + 12A$ and the diploid arm number is $2AN = 52$ (Table I).

The karyotypic constitution of *Trypoxylon* sp3 ($n = 16$ and $2n = 32$) consists of two pseudoacrocentric A^{MC} chromosomes (pair 16) and 30 acrocentrics (Figure 6A and B). Analysis of the C-banding patterns showed that the constitutive heterochromatin of the A^{MC} chromosomes is present throughout the extension of one of

the arms and practically occupies all of the other arm, except for the terminal region. Except for pair 15, in which only the terminal region of the long arm is euchromatic, heterochromatin is located on the short arm and in the centromeric region in most acrocentric chromosomes (Figures 6B and 8B). The diploid karyotype formula is $2K = 2A^{MC} + 30A$ and the diploid arm number is $2AN = 34$ (Table I).

DISCUSSION

The species *T. nitidum*, *T. lactitarse* and *Trypoxylon* sp1 have the same haploid chromosome number ($n = 15$), but differ in arm number ($AN = 20, 21$ and 30, respectively) (Figures 2, 3 and 4 and Table I). Morphological polymorphism observed in the chromosomes of pair 15 in *T. lactitarse* may be explained by a pericentric inversion in which metacentric chromosomes were converted to submetacentrics or vice versa, or may have been due to a change in the functional centromere (Imai *et al.*, 1988b; Imai, 1991) (Figure 9).

On the basis of the minimal interaction theory, Imai *et al.* (1986, 1988a,b, 1994) have proposed that telocentric chromosomes originating from biarmed chromosomes by centric fission are converted to acrocentrics or pseudoacrocentrics by tandem growth of constitutive heterochromatin, and that this heterochromatin region may contain multiple dormant or inactive centromeres and telomeres that may be reactivated and change chromosome morphology. This mechanism was suggested to explain the observations made in *Myrmecia (pilosula)* (Imai and Taylor, 1989) and

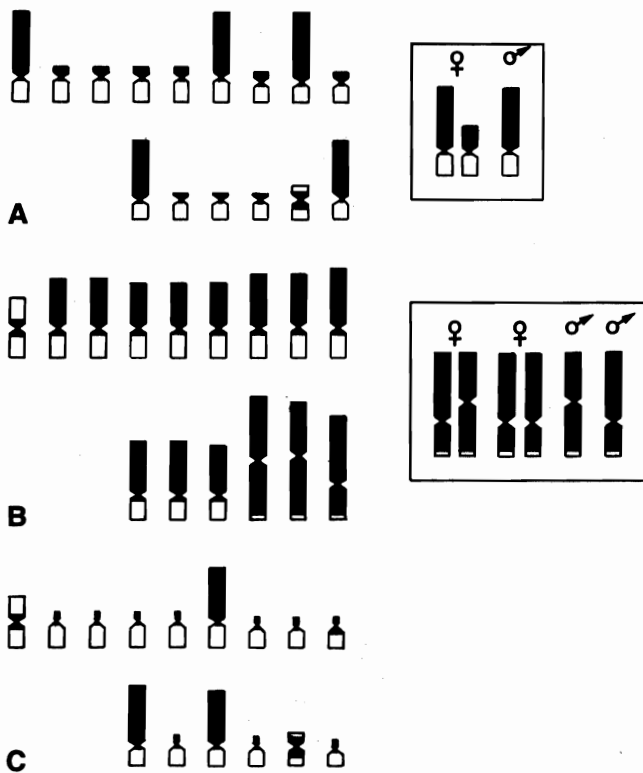


Figure 7 - Idiogram and C-banding patterns. A, *Trypoxylon nitidum* ($n = 15$); B, *Trypoxylon lactitarse* ($n = 15$); C, *Trypoxylon* sp1 ($n = 15$). The insets, contain the types of chromosomes detected in the diploid ($2n$) and haploid (n) karyotypes of *Trypoxylon nitidum* (chromosome 1) and of *Trypoxylon lactitarse* (chromosome 15). The heterochromatin distribution of *Trypoxylon* sp1 is based on the differential staining pattern observed in some metaphases.

Myrmecia (piliventris) (Imai *et al.*, 1988b). Imai (1991) also proposed that the inactivation and reactivation of centromeres is one of the mechanisms by which acrocentric chromosomes with a heterochromatic short arm and proximal region of the centromere of the long arm (A^C) and/or pseudoacrocentric A^{MC} chromosomes arose from pseudoacrocentric chromosomes (A^M). This mechanism may explain the morphology and the patterns of constitutive heterochromatin distribution in the A^{MC} chromosomes that form the karyotype of *T. lactitarse* (Figure 3).

Chromosome 1 of *T. lactitarse* (Figure 3), chromosome 14 of *T. nitidum* (Figure 2) and chromosomes 1 and 14 of *Trypoxylon* sp1 (Figure 4), all of which are metacentrics with heterochromatin in the pericentromeric region (M^{CC}), possibly originated from centric fusions between acrocentric chromosomes.

The species *Trypoxylon* sp3 (Figure 6) ($2n = 32$; $2AN = 34$) differed considerably from the other species studied here in terms of chromosome morphology and size and constitutive heterochromatin quantity and distribution. Its karyotype consists mostly of acrocentric

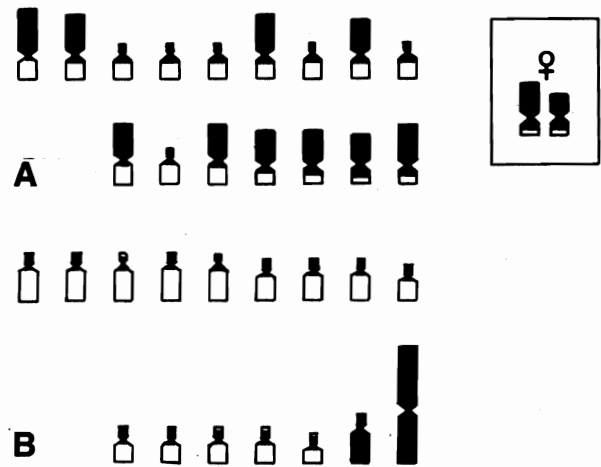


Figure 8 - Idiogram and C-banding patterns. A, *Trypoxylon* sp2 ($n = 16$); B, *Trypoxylon* sp3 ($n = 16$). The inset contains the types of chromosome 14 detected in the diploid ($2n$) karyotypes of *Trypoxylon* sp2. The heterochromatin distribution of *Trypoxylon* sp2 is based on the differential staining pattern observed in some metaphases.

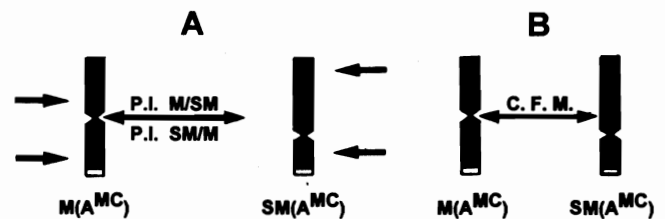


Figure 9 - Schematic presentation of the possible rearrangements involved in the polymorphism of chromosome 15 of *Trypoxylon lactitarse*. A, Polymorphism due to pericentric inversion (P.I.); B, polymorphism due to change in functional centromere (C.F.M.). The regions in black correspond to heterochromatin and the white regions to euchromatin.

chromosomes, except for pair 16 which consists of two large pseudoacrocentric chromosomes (A^{MC}) similar to those observed in the karyotype of *T. lactitarse* (Figure 3). This chromosome may have originated from an acrocentric by tandem growth of constitutive heterochromatin.

The karyotype of *Trypoxylon* sp2 (Figure 5) differs in chromosome number ($2n = 32$) from the remaining species *T. nitidum* (Figure 2), *T. lactitarse* (Figure 3) and *Trypoxylon* sp1 (Figure 4), all of them with $2n = 30$, a lower number which, as mentioned earlier, is attributed to centric fusions. In these four species, the pattern of constitutive heterochromatin distribution is similar in most of the chromosomes presenting the same morphology.

The polymorphism of pair 1 in *T. nitidum* (Figure 2B) and of pair 14 of *Trypoxylon* sp2 (Figure 5) may be explained by translocation, duplication or deletion in one of the homologues. The last possibility seems

to be the most likely since a comparison of homomorphic pairs shows that they are pseudoacrocentrics (A^M), with the heterochromatic arm longer than the euchromatic arm, whereas in the heteromorphic pairs one of the homologues has this proportion but the other has an arm ratio of approximately 1:1, indicating that deletion of heterochromatic material occurred.

The karyotypic constitutions of wasps of the genus *Trypoxylon* analyzed by Hoshiba and Imai (1993), by Gomes *et al.* (1995) and in the present study support the theory of minimal interaction (Imai *et al.*, 1986, 1988a,b, 1994). The variability of diploid chromosome number is very small ($2n = 30$ to 32) and the same variability is observed if only the diploid number of euchromatic arms is considered ($2AN = 30$ to 32). However, if analyzed in terms of diploid arm number ($2AN = 34$ to 60) and chromosome morphology, it can be seen that there is considerable variability (Table I).

In view of these observations and according to the cited theory, we assume that the ancestral karyotype of *Trypoxylon* consisted of seven or eight chromosomes and that evolution occurred in the direction of an increase in chromosome number by centric fission. The existence of an ancestor with a small chromosome number was also suggested by others for different groups of Hymenoptera. Imai (1969) proposed that the more primitive Hymenoptera had a haploid chromosome number of seven to 10, Naito (1978a,b, 1982) proposed $n = 10$ for the ancestral karyotype of Tenthredinidae, and Hoshiba *et al.* (1989) proposed that the ancestral karyotype of Hymenoptera had a chromosome number of 12 or less. These assumptions were made mainly on the basis of the variability in chromosome morphology, number and distribution and content of constitutive heterochromatin.

It was observed that the major agent of karyotype variability in the species studied here both in terms of morphology and of diploid arm number, is the content and distribution of constitutive heterochromatin. The presence of this heterochromatin and its preferential distribution on one of the arms of the chromosome strengthens the assumption of the minimal interaction theory. These observations also support the assumption of the present study that these karyotypes are indeed derived from an ancestral type with a low chromosome number, mainly by centric fission and later growth of constitutive heterochromatin.

Hoshiba and Imai (1993) proposed that the number of chromosomes with a heterochromatic arm increases with increasing chromosome number. These investigators propose that this correlation is a strong indication of the high telomeric instability of telocentric chromosomes in Hymenoptera.

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RESUMO

Neste trabalho são apresentados os cariótipos de cinco espécies de vespas do gênero *Trypoxylon* (subgênero *Trypargilum*) (Hymenoptera, Sphecidae): *T. nitidum* ($2K = 10A^M + 18A + 2\overline{M}^{CC}$), *T. lactitarse* ($2K = 28A^{MC} + 2\overline{M}^{CC}$), *T. sp1* ($K = 3A^M + 10A + 2\overline{M}^{CC}$), *T. sp2* ($2K = 20A^{MC} + 12A$) e *T. sp3* ($2K = 2A^{MC} + 30A$). A comparação entre os cariótipos destas espécies mostrou uma pequena variação quanto ao número de cromossomos ($2n = 30$ e 32). Contudo, uma variação bem maior foi encontrada quando se comparou o número total de braços ($2AN = 34$ a 60). Essa variabilidade é atribuída, principalmente, à diferença na quantidade da heterocromatina constitutiva nos cariótipos das diferentes espécies.

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