Revisiting the karyotype of the social wasp Polistes canadensis (Linnaeus, 1758) (Hymenoptera: Vespidae: Polistinae)

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Abstract

Cytogenetic techniques have been improving over the last decades, providing useful information for the systematics and evolution of several groups, such as social insects. On the other hand, karyotypic data are still incipient for most wasp genera. For instance, only five of the 240 species of Polistes have been karyotyped, usually based on obsolete data. Therefore, this study aimed to revisit the karyotype structure of Polistes canadensis, providing unpublished information based on traditional (karyotyping and C-banding) and refined (GC- and AT-rich sites by base-specific fluorochrome staining) cytogenetic methods. Males and females of P. canadensis were characterized by haploid and diploid numbers of n = 28 and 2n = 56, respectively. The karyotype formula was established in 2K = 18M + 22SM + 16A with a predominance of pericentromeric heterochromatin and terminal GC+ sites in 16 chromosome pairs. Our results differ significantly from the previous karyotype reported for this species, probably related to the utilization of suitable methods of obtaining mitotic chromosomes in the present study. In addition, the detailed analysis of chromosomal microstructure provided potential cytotaxonomic markers for systematic inferences in social wasps.

Introduction

Hymenoptera is a megadiverse order of insects with about 153,000 valid species, comprising wasps, bees and ants (Peters et al. 2017). The social wasps belong to the family Vespidae, being distributed into two subfamilies: Vespinae (67 species from four genera) and Polistinae (more than 1,000 species and 25 genera). The species in the latter are subdivided into the tribes Mischocyttarini (Mischocyttarus with 250 species), Polistini (Polistes with 240 species, 41 of them found in Brazil) and Epiponini (19 genera and 245 species) (Silveira et al. 2021). In Brazil, the Polistinae fauna encompasses 21 genera and nearly 380 described species. Two clades are recognized in the genus Polistes: (1) “New World” wasps, comprising the subgenera Aphanilopterus, Palisotius, Epincemius, Onerarius, and Fuscopolistes distributed throughout Americas and Europe; and (2) “Old World” wasps, including Gyrostoma, Stenopolistes, Nygmopolistes, Megapolistes, Polistella, Sulcopolistes, and Polistes sensu stricto distributed over the Eastern/Indo-Malaysian region (Somavilla et al. 2021).

Even though several biological and ecological analyses have been carried out on social wasps (West-Eberhard 1969; Michener 1974; Wenzel 1998; Nascimento et al. 2008), few cytogenetic reports are available for these insects, totaling only 83 karyotyped species (Marchioro 2019; Menezes et al. 2021), with Polistinae being the most studied subfamily (Menezes et al. 2014). Despite the utility of cytogenetic data for the systematic of distinct taxa, the small size and the high number of chromosomes in insects, besides technological issues, might have hindered the karyotype characterization of most species (Koçak and Okutaner 2017). As a result, various cases of doubtful karyotypic descriptions have been reported (Machida, 1934; Pardi, 1942), as pointed out by Pompolo and Takahashi (1990).

This seems to be the case in Polistes canadensis since the only cytogenetic report is restricted to the determination of haploid number (n = 16) based on obsolete methods (squash technique) with no
information about the chromosomal morphology or banding (Kerr 1952). Taking into account the relevance of karyotypes in systematics and evolutionary inferences in insects (e.g. Okiwelu and Noutcha 2014) and the advances in cytogenetic methods, the goal of the present study was to provide a detailed karyotypic analysis of *P. canadensis*, providing additional information about chromosomal morphology, distribution of constitutive heterochromatin and location of AT/GC-rich sites.

**Methods**

A total of 15 nests of *P. canadensis* were collected from July 2019 to January 2020 at Campus II of the Universidade Estadual do Sudoeste da Bahia in the municipality of Jequié, state of Bahia (13°51'4" S, 40°4'52' W). The adults were stored in 2 mL plastic tubes with 70% ethanol and identified by Dr. Alexandre Somavilla from the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus - AM, Brazil.

Cytogenetic preparations were carried out using the cerebral ganglia of larval in the prepupae stage according to the air-drying technique by Imai et al. (1988), which consists of the fragmentation of cerebral ganglia tissue in a hypotonic solution of colchicine-sodium citrate (0.005%), followed by a serial fixation process in Carnoy's fixative (ethanol: acetic acid in a proportion of 3:1). After air dried, the slides were stained with 10% Giemsa solution in Sörensen phosphate buffer (0.06 M; pH 6.8).

The C-banding technique was performed by Sumner (1972), using the BSG method (barium hydroxide/saline solution/Giemsa), with modifications (Siqueira et al. 2008). For base-specific fluorochrome staining, we used Chromomycin A<sub>3</sub> (CMA<sub>3</sub>), 4'6-diamidino-2-phenylindole (DAPI) to detect GC- and AT-rich sites, respectively, and Distamycin A as a counterstain, as described by Schweizer (1980).

A total of 40 slides were analyzed, representing 38 female and 2 male specimens, with an average of five metaphases each. The best metaphases were photographed using a microscope model SOLARIS-T with a portable digital camera model MEKEY. The karyotypes were arranged using Adobe Photoshop CS6.

The chromosomes were organized in pairs in decreasing order of size and classified according to Levan et al. (1964) based on centromere position.

**Results**

The haploid and diploid numbers of *P. canadensis* were equal to n = 28 and 2n = 56 chromosomes, respectively, with a karyotype formula of 2K = 18M + 22SM + 16A (Fig. 1a). The C-banding revealed heterochromatin segments restricted to the pericentromeric region of all chromosomes (Fig. 1b). The base-specific fluorochrome staining technique revealed GC-rich sites (CMA<sub>3</sub><sup>+</sup>) at the terminal position on 16 chromosome pairs (01, 02, 03, 07, 10, 11, 12, 13, 14, 15, 16, 17, 18, 21, 22, and 23), while AT-rich regions were not observed (Fig. 1c).

**Discussion**
Previous reports revealed that variation in the chromosomal number within a single species might take place in Hymenoptera, as observed in bees of the genus *Melipona* and in social wasps of the tribe Epiponini (Francini et al. 2011; Menezes et al. 2013; Menezes et al. 2014; Barbosa et al. 2021). In some cases, distinct chromosomal numbers refer to the presence of cryptic forms or a species complex, particularly common in groups of a wide geographic range, as reported in wasps of the genera *Metapolybia* (Menezes et al. 2013) and *Polybia* (Marchioro 2019).

For instance, karyotypic variation has been already indicated in *Polistes apachus*, but the author suggested further detailed analysis to confirm the chromosomal number of this species (Machida 1934). Similarly, the present results obtained in *P. canadensis* (n = 28) also differ significantly from the previous report by Kerr in 1952 (n = 16), since we identified 12 additional chromosome pairs in this species. Most likely, this divergence is derived from technical artifacts since the former report was based on squashed cells instead of air-drying technique, as presently performed.

In fact, squash techniques might be harmful to chromosomes either by the rupture of sister chromatids or poor spreading of chromosomes, eventually leading to difficulties in counting chromosomes and defining their morphology (Koçak and Okutaner 2017). On the other hand, as presently performed, air-drying techniques provide reliable cytogenetic results, allowing a refined analysis of chromosomal microstructure. Corroborating the reliability of the present data, the 2n value herein reported for *P. canadensis* is close to the average value in other species of the subgenus *Aphanilopterus* (Pompolo and Takahashi 1986, 1990).

Even though the karyoevolutionary trends in species of *Polistes* remain largely unclear by the lack of additional information, the presence of acrocentric pairs, also reported in other species of *Polistes* (Pompolo and Takahashi 1990) should derive from fissions of metacentric chromosomes, giving rise to unstable one-armed chromosomes (Imai 1991). To counteract such karyotype instability, most acrocentric chromosomes would have undergone heterochromatinization on breakage points, thus determining the appearance of pseudoacrocentric or acrocentric chromosomes (Hoshiba and Imai 1993). Therefore, the presence of conspicuous heterochromatin blocks might mitigate putative telomere instability after centric fissions in species with derived karyotypes characterized by higher 2n values (Imai et al. 1988; Pompolo and Takahashi 1990).

Unfortunately, data about the distribution and composition of heterochromatin are rarely reported in *Polistes*, jeopardizing comparative results among congeneric species. Nonetheless, the C-banding pattern described in this study (heterochromatin at pericentromeric regions) is similar to that reported in other hymenopterans such as Meliponini bees and other social wasps (Vespidae) (Travenzolli 2018; Marchioro 2019).

In addition, the staining with base-specific fluorochromes revealed CMA$_3^+$ signals at euchromatic regions of several chromosomal arms, indicating these regions are GC-rich (Fig. 1c). A similar pattern was found in other species of the genera *Mischocyttarus* and *Polybia* (Menezes et al. 2014; Cunha et al.
suggesting this feature is shared among these taxa and Polistes. According to Menezes et al. (2013), these regions play a key role in some groups of social wasps, once they are supposed to be involved in chromosomal rearrangements. Moreover, GC-rich sites are usually interspersed with 45S rDNA regions, and previous reports associated multiple ribosomal cistrons with the occurrence of centric fissions in Hymenopterans (Menezes et al. 2021). These data reinforce that fissions are actually involved in the speciation process of _P. canadensis_ and further analyses focusing on chromosomal mapping of rDNA genes are highly recommended in this species.

In conclusion, the utilization of additional cytogenetic techniques like C-banding and fluorochrome staining proved to be useful as chromosomal markers to identify, for the first time, the distribution and composition of heterochromatin in the analyzed species. Besides being useful to infer the karyoevolutionary history of _Polistes_ wasps, we reinforce the utilization of cytogenetic data as an additional tool to the systematics of insects by providing a fast characterization of species-specific patterns of genome organization.

### Abbreviations

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<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>2n</td>
<td>Diploid chromosome number</td>
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<tr>
<td>A</td>
<td>Acrocentric chromosome</td>
</tr>
<tr>
<td>AT</td>
<td>Adenine-Thymine</td>
</tr>
<tr>
<td>BSG</td>
<td>Barium hydroxide/Saline solution/Giemsa</td>
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<tr>
<td>CMA₃</td>
<td>Chromomycin A₃</td>
</tr>
<tr>
<td>DAPI</td>
<td>4‘6-diamidino-2-phenylindole</td>
</tr>
<tr>
<td>GC</td>
<td>Guanine-Cytosine</td>
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<tr>
<td>M</td>
<td>Metacentric chromosome</td>
</tr>
<tr>
<td>n</td>
<td>Haploid chromosome number</td>
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<tr>
<td>SM</td>
<td>Submetacentric chromosome</td>
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### Declarations

#### Acknowledgments

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#### Author Contribution

JCSJr and TSP conceived and designed the research. JAB, TSP, RJS, and AOM performed cytogenetic experiments and analyzed data. TSP wrote the manuscript, and JCSJr and JAB
contributed to the definitive version. All authors read and approved the manuscript.

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**Conflicts of interest/Competing interests:** Not applicable.

**Consent for publication:** All authors are aware of and have approved the manuscript prior to submitting it to Chromosoma.

**References**


Figures
Figure 1

Karyotype of *Polistes canadensis*. Conventional staining (female) (a); C-Banding (male) (b); Base-specific fluorochrome staining (female) (c). GC-rich regions are shown in green. Scale = 10 µm