Differences in Karyotypes between Two Populations of Crotalaria incana from Mexico

Fernando Tapia-Pastrana1*, Sandra Gómez-Acevedo2 and Pedro Mercado-Ruaro3

1Division of Postgraduate Studies and Research, Faculty of Higher Studies Zaragoza, UNAM, México
2Unity of Morphology and Function, Faculty of Higher Studies Iztacala, UNAM, México
3Department of Botany Institute of de Biology, UNAM, México

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Summary The karyotypes and NOR condition of the secondary constrictions of chromosome pair 1 in two populations of Crotalaria incana from the Atlantic and Pacific slopes of Mexico are described. Both populations exhibited the same chromosome number of $2n=14$ and karyotypes which shared a secondary constriction on the short arm of chromosome pair 1. Differences were found in the position of the centromere on chromosome pair 5 and in the size of the proximal region of the short arm of chromosome pair 1. In addition, marked differences were observed in total haploid chromosome length (THC) and mean chromosome size (MC), without this affecting asymmetry indexes. Atlantic population exhibited chromosome fragments and fragile sites at prometaphase. These data revealed differences in karyotypes between two populations.

Key words Chromosome size, Cytotype, Fragile site, NOR, Satellite, Karyotype, Crotalaria incana.

Crotalaria L. (Leguminosae) is a tropical genus having more than 600 species described. The genus extends to the subtropics in both hemispheres and, distributed mostly in the east and southeast Africa with an apparent early expansion across the Atlantic and subsequently to Asia and Australia (Polhill 1981, 1982). In America, the secondary diversification center, 71 species have been described (Lewis 1987), with a natural distribution from the south of the United States through Mexico to Brazil, subtropical Argentina and Uruguay (Burkart 1952, Windler 1974, Mondin and Aguiar-Perecin 2011). In Mexico, the genus includes 19 species, eight of which are considered endemic (Sousa and Delgado 1998).

Cytogenetic studies in Crotalaria have offered abundant information in relation to chromosome evolution in the genus. The most notable feature is the chromosome number $2n=16$ observed in all investigated sections, except in the section Chrysocalycinae, subsection Incanae, with $2n=14$ (Boulter et al. 1970, Palomino and Vázquez 1991, Cottias de Oliveira and Aguiar-Perecin 1999, Tapia-Pastrana et al. 2005, Almada et al. 2006). Some tetraploids ($n=16, 2n=32$) and an octaploid ($n=32$) are known from American species of the section Calycinae (Windler 1974, Cottias de Oliveira and Aguiar-Perecin 1999, Almada et al. 2006, Flores et al. 2006, Tapia-Pastrana 2012). It is considered that the basic number in Crotalaria is $x=8$ (Boulter et al. 1970, Gupta and Gupta 1977, Polhill 1982, Mangotra and Koul 1991), while $x=7$ is considered derived (Gupta 1976, Palomino and Vázquez 1991, Mondin and Aguiar-Perecin 2011). Some authors point out that in Crotalaria, the karyotypes show uniformity in size, symmetry and chromosome morphology (Gupta and Gupta 1977, Raina and Verna 1979). However, others point out that, although similar, karyotypes are not particularly uniform and support the view that interspecific karyotypic differences can be used in the characterization of species (Magoon et al. 1963, Chennaveeraiah and Patil 1973, Cottias de Oliveira and Aguiar-Perecin 1999, Almada et al. 2006, Tapia-Pastrana 2012).

C. incana is a pantropical polymorphic species and is distributed from the United States to South America. In Mexico, C. incana is an annual species of wide distribution that inhabits in forests of Quercus, low deciduous forests, xerophilous scrub and is found as ruderal weeds or secondary vegetation in the arid and hot parts of the country. It is found in elevations from a few meters up to 1900 m. It flowers and fructifies from April to December (Villaseñor Ríos and Espinoza García 1998). C. incana exhibits a great adaptive capacity to environmental changes (Stevens et al. 2001), with notable changes in its growth habits from herbaceous to woody, and also due to its morphological variability, particularly in pubescence, hair length, leaflet width, stems, fruits and flower size (Senn 1939, Atchison 1950, Windler et al. 1992, Abdull et al. 2010). This led to the proposal to treat C. incana under the term “C. incana L. sensu lato taxonomic complex” with differences at the infraspecific level (Planchuelo and Carreras 2011).

In this study, we analyzed two populations of C. incana located on opposite oceanic slopes in Mexico, with well-differentiated climatic, geographical and ecological characteristics; in order to deepen cytogenetic knowl-

*Corresponding author, e-mail: pasfer@unam.mx
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edge and verify the probable chromosome remodeling correlated with specialized environments.

Materials and methods

Two populations under study are located in two biological conservation areas belonging to the Instituto de Biología, UNAM. Population EBTLT: The Tuxtlas Tropical Biology Station, a 640 ha reserve located on the Gulf of Mexico slope, to the southeast of the State of Veracruz and to the center of the region called Los Tuxtlas. Population EBCh: The Chamela Biological Station, which occupies a 3319 ha area and is located in the Sierras de la Costa de Jalisco subprovince of the Sierra Madre del Sur Province, on the Pacific slope of Mexico (Table 1). Voucher specimens were deposited at the National Herbarium of the Universidad Nacional Autónoma de México (MEXU).

Seeds were collected in summer 2013 and from at least six plants of each population. Batches of 30 seeds from each plant were used. The seeds were germinated in Petri dishes lined with a moist filter paper at room temperature and under natural light.

Chromosomes at metaphase and prometaphase were obtained following the splash method (Tapia-Pastrana and Mercado-Ruaro 2001) at least 30 root meristems were separated from 0.6–0.8 cm long roots and pretreated with 2 mM 8-hydroxyquinolin for 5 h at room temperature, and fixed in the fixative (ethanol:acetic acid, 3:1). They were then treated with a mixture of 20% pectinase (Sigma) and 2% cellulase (Sigma) in 75 mM KCl for 2 h at 37°C. After centrifugation at 1500 rpm for 10 min, the cell pellet was transferred to 75 mM KCl solution for 20 min at 37°C. After two successive rinses with the KCl solution, they were again fixed in the fixative and subsequently rinsed twice more. One or two drops of the suspension of pellet were placed on clean slides, air-dried and stained in 10% Giemsa (Hycel) for 10 min. Preparations were made permanent using a synthetic resin.

At least ten metaphase plates of intact cells with well-spread chromosomes, no chromosome overlapping, and same contraction and ten prometaphase plates were photographed from each collection, using a microscope Carl Zeiss Axioskope and analyzed for chromosome number determinations. Five photographs of metaphases with chromosomes having similar comparable degrees of contraction and centromeres clearly located were utilized to obtain the THC, MC, the difference in length between the longest chromosome and the shortest chromosome (Range) and the longest/shortest chromosome ratio (L/S). The chromosomes were classified according to Levan et al. (1964) and the index of asymmetry (TF) was obtained following Gupta and Gupta (1977). In addition, cells in prometaphase and interphase were analyzed to corroborate the presence of fragile sites and fragments and verify the number of nucleoli, respectively.

Results

The quantitative characteristics of the karyotypes of both populations are described in Table 2. The plants from both populations show the chromosome number 2n=14 (Fig. 1A, B) and the TF and L/S values exhibited a relative similarity in chromosome morphology (Table 2). Differences were found in the centromere position of chromosome pair 5: in the population EBTLT corresponds to a metacentric pair whereas in the population EBCh corresponds to a submetacentric pair (Fig. 1C). Also, differences were appreciated in chromosome size (Fig. 1C and Table 2). THC, MC, and Range were significantly higher in the chromosome complements of the population EBCh (Table 2). Likewise, differences were observed in the size of the proximal region in the karyotypes of the two populations.

Table 1. Characteristics of the localities considered in this study.

<table>
<thead>
<tr>
<th>Population</th>
<th>Slope coordinates</th>
<th>Elevation (m)</th>
<th>Climate</th>
<th>MAT (°C)</th>
<th>MAR (mm)</th>
<th>Vegetation</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBTLT</td>
<td>Atlantic 18°35’N 95°06’W</td>
<td>150–650</td>
<td>Af(m)</td>
<td>≥22</td>
<td>4725</td>
<td>Tropical rainforest</td>
</tr>
<tr>
<td>EBCh</td>
<td>Pacific 19°30’N 105°03’W</td>
<td>0–150</td>
<td>Aw2</td>
<td>24.6</td>
<td>731</td>
<td>Deciduous forest</td>
</tr>
</tbody>
</table>

MAT=Mean annual temperature, MAR=Mean annual rainfall, Af(m)=Warm-humid tropical ecological zone, Aw2=Subhumid warm tropical ecological zone.

Table 2. Karyotypic analysis of C. incana from the two populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>2n</th>
<th>Karyotype formula</th>
<th>Secondary constriction</th>
<th>THC±S.E (µm)</th>
<th>MC±S.E (µm)</th>
<th>Range±S.E (µm)</th>
<th>L/S±S.E</th>
<th>TF±S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBTLT</td>
<td>14</td>
<td>4n+3sm</td>
<td>1 m</td>
<td>21.96±3.10</td>
<td>3.13±0.30</td>
<td>1.54±0.15</td>
<td>1.63±0.03</td>
<td>40.07±0.82</td>
</tr>
<tr>
<td>EBCh</td>
<td>14</td>
<td>5n+2sm</td>
<td>1 m</td>
<td>27.25±2.12</td>
<td>3.89±0.44</td>
<td>1.94±0.19</td>
<td>1.62±0.04</td>
<td>40.73±0.90</td>
</tr>
</tbody>
</table>
short arm in chromosome pair 1, which is associated with a linear satellite (Fig. 1C). The size of the proximal region of the short arm of chromosome pair 1, in plants from EBCh is longer than that of plants from population EBTLT. In both populations, the secondary constrictions exhibited their NOR activity, and at prophase, the satellites were observed in association with two small nucleoli (Fig. 2A) or with a large single nucleolus (Fig. 2B, C). The maximum number of two nucleoli at interphase (Fig. 3A) confirmed that there are two NORs in the diploid complement.

Only in the population EBTLT all plants had cells whose chromosomes frequently carried fragile sites and fragments probably derived from these in metaphase and prometaphase (Fig. 3B–D).

Discussion

It should be noted that Palomino and Vázquez (1991) reported three or four secondary constrictions in two populations of C. incana, however, in a subsequent investigation, it was shown that this species exhibits uncoiled terminal regions that could be confused with secondary constrictions (Tapia-Pastrana et al. 2005). It exhibits, like most species of the genus, a single secondary constriction in the short arm of the chromosome pair 1, as associated with a locus of 45S rDNA (Mondin and Aguilar-Perecin 2011). The secondary constrictions and satellites adhered to or embedded in the nucleolus at prometaphase (Fig. 2A–C) reaffirm their active participation in the formation of this one.
This study corroborated the cytogenetic characteristics in *C. incana* and proposes two karyotypic formulas for the populations studied here (Table 2), that differ in chromosome size, centromere position in chromosome pair 5, and short arm characteristics in chromosome pair 1. The presence of fragile sites (Fig. 3B–D) might involve translocations, as suggested by Atchison (1950). Fragile sites have been recognized for a long time in metazoans as regions prone to break under replication stress, and their participation in chromosome reorganization in plants is beginning to reveal itself. For example, it is suspected that at the speciation events of *Arabidopsis thaliana* and *A. lyrata* generally unstable regions prone to deletions were involved, as well as other large chromosome rearrangement events (de la Paz et al. 2012).

Previous studies have shown an association between chromosome size and ecogeographic gradients (*e.g.*, *Prosopis*; Tapia-Pastrana et al. 1999, *Prosopis* and *Acacia*; Gómez-Acevedo and Tapia-Pastrana 2003, *Karwinskia*; Tapia-Pastrana et al. 2009). The differences in THC in the *C. incana* populations evaluated in this study suggest the loss of genetic interaction between these two populations.

The *C. incana* populations here analyzed inhabit ecologically differentiated areas: the EBTLT is included in the warm-humid tropical ecological zone, while the EBCt is within the subhumid warm tropical ecological zone in Mexico. Both zones differ strongly in relation to seasonality (Table 1). If it is considered that there is no genetic interaction between these two geographically isolated populations, it is likely that the differences in chromosome sizes and chromosome shape point towards a process of genomic differentiation *via* chromosome evolution that shows the speciation process (Kenton 1981, 1984, Kenton et al. 1988, Grant 1989, Palomino and Martínez 1994, Martínez et al. 2000).

In this study, we show the karyotype differences between these two populations. It might suggest that the difference in karyotype may represent a change associated with speciation. In the future, a comparison of genome size, molecular cytogenetic and molecular phylogenetic analyses are required in order to reveal the genetic differentiation between these populations.

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