Cytogenetics and Evolutionary Analysis of *Lopanthera*, an Amazonian Arboreal Malpighiaceae

Ricardo A. Lombello and Eliana R. Forni-Martins

1 Post-graduating in Vegetal Biology, IB/Unicamp
2 Department of Botany, Biology Institute, State University of Campinas (Unicamp), Caixa Postal 6109, CEP 13083-970, Campinas-SP, Brazil

Received November 5, 2001; accepted December 12, 2001

Summary Only 2 chromosome counts are reported for *Lopanthera*, an Amazonian arboreal genus with 5 species that belongs to sub-family Byrsonimoideae. Cytological studies were carried out in *Lopanthera lactescens* Ducke to verify the haploid and diploid chromosome numbers (*n*=6, 2*n*=12) of a previously unstudied population. The meiotic process was regular and no abnormalities were found. The first chromosomal ideogram for the genus was constructed and shows a predominance of metacentric pairs (5 m+1sm). In order to explain the increased lengths of its chromosomes (5.3 μm to 11.8 μm) and the primitive position of the genus in Malpighiaceae, in situ hybridization with a telomeric probe was performed. We observed the distribution of the telomeric sequences. No interstitial sequences were found. In addition to the symmetric karyotype observed, this assay indicates that probably no fusion or translocations of segments has occurred along the karyotype evolution of *Lopanthera*.

Key words *Lopanthera*, Byrsonimoideae, Cytogenetics, Karyomorphology, In situ hybridization.

The genus *Lopanthera* Ducke, that belongs to family Malpighiaceae, sub-family Byrsonimoideae, tribe Galphimieae, possesses 5 known species, 4 solely found in the Amazonian region (Anderson 1977), and 1 occurring in Costa Rica, Central America (Anderson 1983). These are plants with shrubby or arboreal habits and an elongated yellow inflorescence. Because of its less derived characters, such as arboreal habit, leaf glands, tyriform inflorescence, 5 sepals with 2 abaxial glands and tricolpate pollen grains (Anderson 1977, 1981, 1983), the genus *Lopanthera* is accepted as one of the most primitive within Malpighiaceae.

Two species of *Lopanthera* present chromosome number reports: *L. lactescens* and *L. hammeli*, both *n*=6 (Lewis and Oliver 1970, Anderson 1981). The cytological characters also support the supposed primitivism of *Lopanthera*. Together with *Galphimia*, another genus of tribe Galphimieae, *Lopanthera* presents the smallest chromosome number within Malpighiaceae, which presents counts, as that of *Banisteriopsis stellaris* (Lombello 2000), ranging up to 2*n*=80. *Galphimia* is the genus of Malpighiaceae that presents the largest chromosomes (Zaman et al. 1977, Lombello 2000). Considering the diversity of chromosome numbers and of chromosome lengths reported for the family (Lombello 2000), the karyomorphological analysis became important to discuss the chromosomal evolution within Malpighiaceae.

The DNA in situ hybridization allows a localization of specific sequences in metaphase chromosomes, leading to construction of detailed chromosome ideograms, as those presented by Leitch and Heslop-Harrison (1992) for barley (*Hordeum vulgare*). We used this technique in order to identify the presence and distribution of telomeric sequences in chromosomes of *L. lactescens*.

Telomeres are important structural and functional elements of chromosomes. They are composed of short sequences, usually TTTAGGG, repeated in tandem (Adams et al. 2000), polymerized by telomerase (Kilian et al. 1995). Acting as an in vivo protection cape of chromosomes,
telomeres prevent the degradation of terminal portions of chromosomes by nucleases (Wang and Lapitain 1992). They inhibit chromosome fusion and the shortening of linear DNA caused by incomplete replications at 5' termination by DNA polymerase (Fuchs et al. 1995). The shortening or losing of telomeres are 2 of the most important causes of Robertsonian fusion, where 2 acrocentric chromosomes bind to form a metacentric one (Slijepevic 1998). Moreover, this fusion is closely related to cellular senescence (Kilian et al. 1995).

We have chosen to use a telomeric probe because the presence of extreme sequences in interstitial positions possibly indicates the occurrence of breaks or fusion in chromosomes. It can also indicates the presence of breaking sites, where recombination and rearrangement may occur during the evolutionary process of species, as pointed out by Schwarzacher and Heslop-Harrison (1991) for *Hordeum vulgare*. Studies of telomere localization may be useful in evolutionary discussion inside groups.

The present paper seeks to analyze a population of *L. lactescens* different from that studied by Anderson (1983), to obtain karyomorphological data for the species and to investigate possible structural chromosome alterations involved in karyotype derivation in Malpighiaceae.

**Materials and methods**

*Plant material and standard chromosome preparations*

We studied seeds and floral buds of individuals cultivated at Unicamp campus, Campinas-SP, Brazil. Vouchers are deposited at UEC herbarium, identified as Lombello 048. Roots were pre-treated with PDB in saturated solution for 4 h at 16°C and fixed in Carnoy's solution (alcohol 3 : 1 glacial acetic acid, v/v). For mitosis analysis root tips were hydrolyzed in 5 N HCl and squashed in 45% acetic acid. A 2% Giemsa solution was used in slide staining. For ideogram confection, 10 metaphases were draught in camara lucida and their chromosomes were measured. For the meiosis analysis, authors fixed in Carnoy's solution were squashed with 1.2% acetic carmine solution. We observed 10 pollen mother cells and 435 pollen grains.

*In situ hybridization*

For the *in situ* hybridization we used pre-treated root tips digested in a mix of citrate buffer with 2% of cellulase and 20% of liquid pectinase for 1 h at 37°C. These roots were squashed in 45% acetic acid and the slides were stored for 1 week at last. The slides were washed with RNase and the hybridization assay followed the protocol of Viegas-Péquiont (1992). The probe used was pLT11 with a telomeric insert of pAtT4 from *Arabidopsis thaliana* (Richard and Ausubel 1988), labeled with biotin by nick translation, detected with IgG-FITC and counterstained with propidium iodide. The observations were made in Zeiss fluorescence photomicroscope, registered in Kodak multispeed 400 ASA film.

**Results**

The haploid and diploid chromosome number *n*=6 and 2*n*=12 were observed for *L. lactescens* (Fig. 1a, b). Karyomorphological characters such as chromosome lengths and centromeric position were used to construct the ideogram (Fig. 2). The chromosomes are the largest observed within Malpighiaceae, varying from 5.3 μm to 11.8 μm (Table 1, Fig. 1b). The chromosome length variation is gradual (Fig. 2). The karyotype formula in 5m+1sm. A secondary constriction in the long arm was observed in pair 3 (Figs. 1b, 2). The total chromatin length is 101.3±9.3 μm and the TF% (Huziwara 1962) index is 44.8. The chromosome pair classification and centromeric index for each pair is given in Table 1. In meiotic process analysis no abnormalities were found. Six bivalent ones were observed (Fig. 1a). The viable pollen index was high (93.3%).
The in situ hybridization with telomere probe pLT11 evidenced the extreme regions of almost all the chromosomes (Fig. 1c), although the signal intensity was different between chromosomes and even arms. No interstitial sites have been evidenced in the chromosomes.

Discussion

Chromosome number

The chromosome counts $n$ and $2n$ agree with the only 2 presented in literature for L. lactescens (Lewis and Oliver 1970, Anderson 1981) and L. hammelii, with $n=6$ (Anderson 1981). This is the smallest chromosome number ($2n=12$) registered for a Malpighiaceae species. Anderson (1983) suggests that the basic number $x=6$ is the least derived in the family considering that for Galphimia glauca, a Lophanthera closely related species, the chromosome basic number is the same (Seavey 1975). Both genera belong to the sub-family Byrsonimoideae, considered the most primitive group in the family.

The basic number $x=6$ also occurs in other families of Malpighiales order, as Linaceae, Passifloraceae and Violaceae (Raven 1975, Watson and Dallwitz 1992), which reinforces its primitive character.

Karyomorphological data

Another character that probably indicates a less derived position of L. lactescens is the relatively large size of its chromosomes ($5.3–11.8\mu m$) when compared with those presented for other
species of Malpighiaceae. For *Mascagnia anisopetala* (Lombello and Forni-Martins 1998) presented chromosomes varying from 1.1 to 2.4 μm. For *Hiptage benghalensis*, Devar and Boraiah (1981) presented chromosomes with 1.62 to 3.25 μm. In addition to their lengths, the predominance of metacentric pairs and the symmetrical karyotype pattern indicate a less derived cytogenetic feature, as pointed out by Stebbins Jr. (1971). These are first karyomorphological data presented for the genus *Lophanthera*. For *Galphimia*, a *Lophanthera* related genus, we found karyomorphological reports for 2 species. For *Galphimia gracilis*, Zaman et al. (1977) showed relatively large chromosomes, with lengths ranging from 5.1 to 9.4 μm, with predominance of metacentric ones (9m+3sm). For *Galphimia gracilis* (Zaman et al. 1977) presented a secondary constriction on a long arm of one chromosome pair, as observed here for *L. lactescens*. For *Galphimia brasiliensis*, Lombello (2000) presented chromosomes that vary between 4.0 and 8.2 μm, with predominance of metacentric pairs (11m+1sm). These data lead us to presume that increased chromosomes is a pattern of sub-family Byrsonimoideae.

**Telomeric sequences distribution**

We did not observe any telomeric sequences in interstitial sites during the hybridization assay. Interstitial telomeric sequences occur in about half of vertebrate studied species, although they are not very common in plants (Fuchs et al. 1995). One of the most important causes of Robertsonian fusion, structural or functional alterations in telomeres are frequently involved with mammalian karyotype evolution (Slijepcevic 1998). Still, this is not the main process in karyotype evolution of plants (Fuchs et al. 1995), which is polyploidy (Stebbins Jr. 1971).

Variation in intensity of signals obtained from *in situ* hybridization, like those observed here, are common. Ganal et al. (1991) also observed that the signal varies between the ends of individual chromosome pairs of tomato. Guerra and Kenton (1996) showed that in the hybrid *Phaseolus vulgaris*×*P. acutifolius*, some chromosomes did not present any signal at any extremity. This absence may be due to an hybridized probe concentration below the threshold of detection (Guerra and Kenton 1996), or to low repeated sequences.

**Karyotype evolution**

The karyotype evolution may be regarded as increasing or decreasing both the chromosome number and its length (Stebbins Jr. 1971). Since probe pLT11, specific to telomeric regions, evidenced only extreme sites, it probably indicates that the relatively large chromosomes of *L. lactescens* did not originated from chromosomal fusion, numeric reduction and breaks and fusions of chromosome segments of relative species. In other words, this increased size of *L. lactescens* chromosomes, as well as that reported for *Galphimia* species, is probably a less derived character, which means the beginning of an evolutionary sequence, supported by the morphological characters. In the present study the *in situ* hybridization with telomeric probe supports the hypothesis that increased length of chromosomes is a less derived condition in Malpighiaceae family.
Acknowledgements

The authors are grateful to Dr. Shirley M. Recco-Pimentel from Department of Cell Biology of Unicamp for suggestions and lending her laboratory structure. We also thank Dr. Maria C. Mamede from Instituto de Botânica de São Paulo for plant identification and CAPES for financial support.

References