Chromosome variations and diversity of *Epidendrum ibaguense* Lindl. (Orchidaceae) on the Tepequém’s Tepuy, Roraima, Brazil

S.R. Nóbrega¹, A.L.F. Coelho², C.F. Verola³, I.R. Costa³, R. Vilaça¹, F.J.F. Luz⁴ and W.F. Araújo¹

¹Rede de Biodiversidade e Biotecnologia da Amazônia Legal, Centro de Ciências Agrárias, Universidade Federal de Roraima, Boa Vista, RR, Brasil
²Programa de Pós-Graduação em Ensino de Ciência e Matemática, Universidade Federal do Ceará, Fortaleza, CE, Brasil
³Departamento de Biologia, Universidade Federal do Ceará, Fortaleza, CE, Brasil
⁴Embrapa, Boa Vista, RR, Brasil

Corresponding author: S.R. Nóbrega
E-mail: sannaro cha@gmail.com

Genet. Mol. Res. 16 (3): gmr16039754
Received June 20, 2017
Accepted August 14, 2017
Published September 21, 2017
DOI http://dx.doi.org/10.4238/gmr16039754

Copyright © 2017 The Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution ShareAlike (CC BY-SA) 4.0 License.

ABSTRACT. Studies addressing chromosome variations have elucidated many points regarding the taxonomy of the Orchidaceae. *Epidendrum* L. besides being one the largest orchid genera, present remarkable morphological, and inter- and intraspecific chromosome variations. Thus, based on a previous report on flower color variation in individuals of *E. ibaguense* (magenta, pink, white, and red), our aim was to determine its chromosome number and test whether this trait is associated with flower color variation in natural populations on the Tepequém’s Tepuy, Roraima. Root apices were pre-treated with 8-hydroxyquinoline at 4°C for 24 h and subsequently submitted to conventional cytogenetic procedures. Slides with the best spreading and contraction of chromosomes were photographed under light
microscopy. Chromosome number was determined by counting at least 10 mitotic metaphase cells per individual. The types of interphase nuclei were determined for 30 nuclei per individual. *E. ibaguense* presented intra- and interpopulation variation in chromosome number, with $2n = 58$, $72$, and $76$. The chromosome number $2n = 58$ was most commonly found in individuals with magenta, pink, and white flowers, while the remaining two chromosome numbers occurred mostly in red-flowered individuals. The types of interphase nuclei were associated with the chromosome number. Individuals with $2n = 58$ presented a predominance of semi-reticulated nuclei, while in those with $2n = 72$ and 76 the nuclei were predominantly non-reticulated. The dominance of disploidy in *E. ibaguense* suggests that this cytotype provides this species with a territorial advantage and a higher reproductive success, possibly contradicting the polyploid hypothesis. Our results suggest that chromosome number may not represent a reproductive barrier in genus *Epidendrum*.

**Key words:** Chromosomes; Hybridization; Cytogenetics; Amazon; Orchids

**INTRODUCTION**

Chromosome analyses provide strong support for plant biosystematics, especially when the variation is a key trait that helps to recognize the validity of a certain species (Stace, 1991). According to Guerra (1986) and Stace (1991), the divergence in chromosome number between morphologically close species can split distinct taxa, remarkably when the morphological variation is also present.

Thus, studies addressing chromosome variations have undoubtedly shed light into taxonomic statuses of the Orchidaceae, one of the largest Angiosperm families. Among the many genera composing this family, *Epidendrum* L. stands out with about 1500 described species (Chase et al., 2003). In *Epidendrum*, chromosome number can vary greatly, ranging from $2n = 24$ in *E. fulgens* Brongn. to $2n = 240$ in *E. cinnabarum* Salzm. Despite such variation, chromosome number is currently known for only 3% of the *Epidendrum* species. Apart from these interspecific differences, chromosome number varies within a given species, and even between neighboring populations (e.g., *E. denticulatum* Barb., $2n = 38, 40$; *E. radicans* Pav. ex Lindl. Rodr., $2n = 40, 57, 60, 62, 64, 70$; and *E. xanthinum* Lindl., $2n = 28, 30, 40, 60$, ca. 80) (Pinheiro et al., 2009; Felix and Guerra, 2010; Assis et al., 2013). The largest number of cytotypes is currently known for *E. secundum* Jacq., with $2n = 28, 40, 48, 52, 68$, and 80 (Pinheiro et al., 2009) or $2n = 30, 42, 50, 54, 56, 58$, and 84 (Assis et al., 2013). Additionally, in *E. secundum* the chromosomes also vary in size, revealing a strong karyotypic asymmetry and bimodality (Assis et al., 2013).

When compared to dysploidy, polyploidy is considered a major adaptive mechanism in plants, expanding their ecological and geographic distribution (Briggs and Walters, 1997). Along with hybridization, polyploidy is recognized as a key process in plant speciation (Rieseberg, 1997; Grant, 1981). Studies suggest that polyploid organisms, which have more than two genomes in the same nucleus, have increased adaptability to harsh environmental conditions, as well as being good colonizers (Ramsey and Schemske, 1998; Schifino-Wittmann, 2004; Fawcett and Van de Peer, 2010). Moreover, polyploidy is apparently related
Chromosome variations and diversity of *E. ibaguense* to subspecific morphological variation (Pinheiro and Cozzolino, 2013). In *Epidendrum*, besides chromosome number variations, morphological traits of the species also vary greatly (Dressler, 1993, 2005; Pinheiro and Barros, 2007; Pansarin and Amaral, 2008). *E. ibaguense* Kunth. is distributed from Mexico to Bolivia, and is commonly found in rocky outcrops, at elevations ranging from 200 - 1000 m, both in the savannas and Rain forests (Freitas, 2001; Luz and Franco, 2012; Flora do Brasil 2020 em construção, 2017). In Brazil, its distribution is limited to the North and Midwest regions, within the Amazon Domain (Tropicos.org domain, 2017; Flora do Brasil 2020 em construção, 2017). Individuals of *E. ibaguense* with different flower colors were reported on a “Tepuy” (Luz and Franco, 2012). This is a type of ancient, table-top Mountain with a particular flora (Alves et al., 2007). Based on this report, and given the great variation in chromosome number in genus *Epidendrum*, this study aimed 1) to determine the chromosome numbers, and 2) to test for an association between chromosome number and flower color variation in natural populations of *E. ibaguense* on the Tepequém’s Tepuy, municipality of Amajari municipality, Roraima.

**MATERIAL AND METHODS**

**Study site**

We collected individuals of *E. ibaguense* from the Tepequém’s Tepuy, in the municipality of Amajari/RR. The Tepequém’s Tepuy is a remain of an eroded sandstone plateau, reaching up to 1100 m. Its flora is composed mainly of grasses, shrubs, and subshrubs (Silva, 1997). The studied populations of *E. ibaguense* are located at the following coordinates: 3°45'56.96750"N and 61°42'09.59398"W, at 831 m (population 1), and 3°45'55.34681"N and 61°41'18.97437"W, at 1056 m (population 2).

**Sample selection and collection**

To determine the chromosome numbers of *E. ibaguense*, we selected individuals with different flower colors: magenta and pink (the most common individuals), and red and white (rare individuals) (Figure 1). The morphological characterization of vouchers used in the cytogenetic analysis is shown in Table 1.

![Figure 1. Flower color variation of Epidendrum ibaguense on the Tepequém’s Tepuy/RR. A. magenta flowers; B. pink flowers; C. white flowers; D. terrestrial red flowers from population 1 (1); E. rupiculous red flowers from the population 2 (2).](image-url)
Chromosome analysis

We previously treated root apices of *E. ibaguense* with 8-hydroxyquinoline at 4°C for 24 h, and postfixed the samples in Carnoy (ethanol:acetic acid, 3:1) at 4°C. The root apices were hydrolyzed in 1 N HCl for 10 min at 60°C, smashed on glass slides, and stained with 2% Giemsa for 20 min (adapted from Guerra, 1988). We photographed those slides with the best spreading and contraction of chromosomes under a light microscope. We determined the chromosome number for at least 10 mitotic metaphases per individual, and the type of interphasic nuclei in 30 samples per individual.

Table 1. Morphological traits of the vouchers used in the cytogenetic analysis.

<table>
<thead>
<tr>
<th>Population</th>
<th>Magenta</th>
<th>Pink</th>
<th>White</th>
<th>Red (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf length</td>
<td>59.41</td>
<td>55.38</td>
<td>60.05</td>
<td>54.2</td>
</tr>
<tr>
<td>Leaf width</td>
<td>2.63</td>
<td>2.4</td>
<td>1.87</td>
<td>1.81</td>
</tr>
<tr>
<td>Inflorescence length*</td>
<td>65</td>
<td>113.75</td>
<td>78.5</td>
<td>36</td>
</tr>
<tr>
<td>Flower /Inflorescence</td>
<td>5F 8B</td>
<td>5F 8B</td>
<td>5F 9B</td>
<td>5F 6B</td>
</tr>
<tr>
<td>Sepal length</td>
<td>11.75</td>
<td>13.85</td>
<td>16.22</td>
<td>14.03</td>
</tr>
<tr>
<td>Petal length</td>
<td>16.42</td>
<td>13.26</td>
<td>17.44</td>
<td>13.77</td>
</tr>
<tr>
<td>Labellum length</td>
<td>19.7</td>
<td>18.99</td>
<td>18.13</td>
<td>19.27</td>
</tr>
<tr>
<td>Labellum diameter</td>
<td>14.94</td>
<td>11.36</td>
<td>12.51</td>
<td>11.47</td>
</tr>
<tr>
<td>Column length</td>
<td>9.49</td>
<td>8.69</td>
<td>11.95</td>
<td>9.07</td>
</tr>
<tr>
<td>Habit</td>
<td>Terrestrial</td>
<td>Rupicolous</td>
<td>Terrestrial</td>
<td>Terrestrial</td>
</tr>
</tbody>
</table>

Values are reported in millimeters, *except for inflorescence length, which is in centimeters. F- Flowers and B- Buds.

RESULTS

*E. ibaguense* presented intra- and interpopulation variation in chromosome number, with 2n = 58, 72, and 76 (Figure 2), and 2n = 58 being most common in four individuals from both studied populations. This finding represents a valuable contribution to the cytogenetic status of *E. ibaguense* since 2n = 70 has been the only chromosome number report up to now (Pinheiro et al., 2009). At population 1, the terrestrial, magenta-flowered individual presented 2n = 58, while the terrestrial, red-flowered individual sheltered in the shade presented 2n = 72. At population 2, the rupiculous individuals with magenta, pink, and white flowers presented 2n = 58, while rupiculous, red-flowered individuals exposed to the sunlight presented 2n = 76. Moreover, in individuals with 2n = 58, interphasic nuclei were predominantly semi-reticulated, whereas in those with 2n = 72 and 76 nuclei were predominantly non-reticulated.

Figure 2. Metaphasic cells of *Epidendrum ibaguense* showing condensed chromosomes. A. 2n = 58, magenta-flowered morphotype; B. 2n = 72, terrestrial, red-flowered morphotype (population 1); C. 2n = 76, rupiculous, red-flowered (population 2). Type of interphasic nuclei: D. semi-reticulated; E. non-reticulated.
DISCUSSION

In *E. ibaguense*, the chromosome number was first reported by Pinheiro et al. (2009). These authors determined $2n = 70$ for individuals from Serra de Pacaraima, located on the border between Venezuela and Roraima. In our study, in both studied populations, $2n = 58$ was the most frequent chromosome number, while $2n = 72$ and $2n = 76$ were less frequent. Therefore, in both populations of the Tepequêm’s Tepuy, diploid individuals of *E. ibaguense* ($2n = 58$) may have greater reproductive and environmental success, as suggested by their higher local abundance. Our results suggest that in *E. ibaguense*, descendant and ascendant diploid events may have caused the chromosome variation in this species on the Tepequêm’s Tepuy.

According to Felix and Guerra (2010), in the subfamily Epidendroideae, only five of the 128 genera present a stable number of chromosomes. *Epidendrum* is one of the few genera that present variation in chromosome number, including at the intraspecific level [e.g., *E. ciliare* L., *E. denticulatum*, *E. radicans*, *E. xanthinum*, and in the complex *E. secundum* (Tanaka and Kamemoto, 1984; Pinheiro et al., 2009; Felix and Guerra, 2010; Assis et al., 2013) and *E. ibaguense* (this study)].

Such variation in chromosome number in *Epidendrum* suggests that determining a basic karyotype for this genus is uncertain, bringing about the difficulty in estimating the level of polyploidy and the karyotypic evolution in this taxon (Felix and Guerra, 2000). However, dysploidy (loss or gain of chromosomes) has been considered the most important evolutionary events in Orchidaceae (Felix and Guerra, 2010), while in plants as a whole, as well as in many orchid genera, polyploidy plays such a role (Felix and Guerra, 2000, 2005; Conceição et al., 2006).

It has been observed that highly polymorphic species are adapted to environmental conditions that impose strong selective pressures. Some groups within Orchidaceae that grow on inselbergs show levels of polyploidy higher than those related to epiphytic species (Felix and Guerra, 2010). In *E. cinnabarina*, for instance, the intraspecific polyploidy seems to be related to the acquisition of the rupiculous habit (Felix and Guerra, 2010). Interestingly, this and other polyploid species are found in disturbed environments (Assis, 2009; Pinheiro et al., 2009; Felix and Guerra, 2010). Duplicated or repetitive gene sequence may provide a local advantage for polyploid organisms (Fawcett and Van der Peer, 2010). On the other hand, such a variation in chromosome number may not represent an effective reproductive barrier neither for *Epidendrum* nor the remaining groups of plants (Cozzolino et al., 2004; Marques et al., 2010).

*E. ibaguense* is phylogenetically close to the Andean clade, mainly distributed between the Andean Mountains and Guyana (Pinheiro et al., 2009; Pinheiro and Cozzolino, 2013). In Brazil, it is found exclusively in the Amazon, particularly on the studied Tepuy. Little is known about these types of mountains, and biological information concerning this orchid group is important to understand its evolution.

Flower color variation in *E. ibaguense* may be associated with the attraction of the different local groups of pollinators (Proctor and Yeo, 1973; Richards, 1996). In the Amazonian orchids, the most common flower colors are yellow, white, and lilac, which lie within the visible spectrum of bees (Ribeiro et al., 1999). Red flowers are invisible to bees and are uncommon in orchids (Faegri and Van Der Pijl, 1979). According to Weiss (1995), flower color change evolved in response to a pollinator-driven selection, which may represent a functional convergence in the angiosperms. Red-flowered individuals of *E. ibaguense* may be coevolving with a specific group of pollinators.

There are still knowledge gaps concerning Amazonian Orchidaceae, and, thus, much...
to be discovered about this family (Krahl et al., 2015). Moreover, studies addressing the origins of the tremendous diversity of *Epidendrum* are often constrained by the lack of model organisms capable of answering many fundamental questions about plant evolution (Pinheiro and Cozzolino, 2013). Therefore, punctual studies may fill such a gap about this orchid genus.

**Conflict of interest**

The authors declare no conflicts of interest.

**ACKNOWLEDGMENTS**

We thank to CAPES and FAPEAM for Doctorate scholarship for S.R. Nóbrega. This study is part of Doctorate’s thesis of S.R. Nóbrega at Network Biodiversity and Biotechnology of the Legal Amazon, UFRR. FUNCAPI - Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (Grant #10582430-5 - Programa Primeiros Projetos) and CNPq to Itayguara Ribeiro da Costa (#479263/2011-6 - Edital Universal 2011). Authorization SISBIO #40111-1.

**REFERENCES**


Chromosome variations and diversity of E. ibaguense


Genetics and Molecular Research 16 (3): gmr16039754