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Research Article

The Yellow Cardinal (Gubernatrix Cristata), An Endangered Species, Is Organized By Karyotype.

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Abstract

There are numerous uses for karyotypic analysis in cytotaxonomy, evolution, and chromosome organization research. Additionally, they are necessary for genome assembly initiatives. Here, we use traditional staining with Giemsa and 18S rDNA probes to describe the karyotype of the endangered yellow cardinal, Gubernatrix cristata (Passeriformes, Thraupidae), for the first time.12 pairs of macrochromosomes and 27 pairs of microchromosomes make up this species' 78 chromosomes. Four microchromosomes included the 18S rDNA clusters. Our findings showed that G. cristata had about 80 chromosomes, which is a normal avian karyotype. However, because the ancestral condition only corresponds to two microchromosomes with these sequences, G. cristata possesses an apomorphic state with respect to the distribution of 18S rDNA. The number of 18S rDNA clusters in G. cristata was most likely increased by duplications and translocations. The findings were contrasted and examined in relation to other members of the Passeriformes and Thraupidae families. Given that G. cristata is classified as a globally threatened species, we think that describing its karyotype could serve as a foundation for upcoming cytogenetics and sequencing initiatives.

Keywords : *rDNA, avian chromosomes, genome, and Thraupidae.*

INTRODUCTION

Avian cytogenetics is a relatively recent field. The karyotypes of just 9.83% of bird species have been described to date [1]. Even though the order Passeriformes has the most karyotyped species (460), this number only accounts for 7% of the order's species [1], suggesting that little is known about the chromosome organization and evolution in this group. Of the roughly 380 species in the Thraupidae family, 11.8% have karyotypes described [1]. For many years, karyotypic investigations have been employed in research on the organization, evolution, and cytotaxonomy of birds' chromosomes [2,3]. The number of chromosomes discovered during genome sequencing and assembly can also be verified using the karyotypes.

For example, whole-genome shotgun sequencing revealed that the canary (Serinus canaria) had 35 (2n = 70) assembled chromosomal groups; however, cytogenetic analysis using traditional Giemsa staining revealed that the proper number of chromosomes is 40 (2n = 80) [4]. The high GC content [5], tandem repeats [6], and numerous microchromosomes [4] in avian species are the causes of this discrepancy. These results emphasize how crucial karyotype description is to proper chromosomal assembly.

The Tharaupidae (Passeriformes, Oscines) includes the yellow cardinal (Gubernatrix cristata). Because of its ongoing population reduction, the species is considered endangered and threatened worldwide [7]. The primary concerns include trapping, particularly of males, and habitat loss, primarily as a result of native fields being turned into agricultural regions [8–10].for the illicit bird trafficking [10–12].

The species is found in southern South America's Pampa Biome. It is closely linked to savanna-park-type vegetation, which may be found in several regions of Argentina, Uruguay, and a few locations in Brazil's Rio Grande do Sul State [13–16]. Throughout its range, G. cristata has always been uncommon; at the moment, records are few and limited to locations that are either legally protected or difficult to reach [17–19]. This is the situation in Brazil, where Espinilho State Park and the adjacent surroundings are home to the sole population,

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which consists of roughly 50 specimens [20,21].

The species was listed in a Brazilian national action plan for the conservation of endangered passerines of the Campos Sulinos because of the degree of threat it poses. One of the objectives of this action plan is to survey the bird populations and genetic data and use the findings to enhance management and conservation plans in the area [20]. G. cristata has not been the subject of any prior cytogenetic investigations. Therefore, we have described the karyotype of this species in this study, including the distribution of 18S rDNA clusters. Our goal was to present this endangered species' cytogenetic data, which could be helpful for upcoming research, particularly genome assembly. Furthermore, we have contrasted our findings with those of others.

Materials and Methods

Animal

In this investigation, a single male G. cristata individual was employed. In 2014, the animal was kept at Porto Alegre, Rio Grande do Sul State's Animal Screening Center (Centro de Triagem de Animais Silvestres). The "Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais" submitted the animal to the Center after it was caught during one of their inspection missions.

Description of Cell Culture and Karyotype

Feather pulp from the male G. cristata individual was used to create a cell culture after [22]. In short, type IV collagenase and mechanical dissociation were used for an hour to separate the feather pulp. Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% fetal bovine serum, 2% penicillin streptomycin, and 1% L-glutamine was used to cultivate the obtained cell suspension at 37 °C. Following a 1-hour colcemid treatment, a 15-minute hypotonic solution (0.075 M KCl), and a 3:1 methanol/acetic acid fixing, metaphase chromosomal spreads were obtained. In at least 20 metaphase plates stained with 10% Giemsa in 0.07 M phosphate buffer, at pH 6.8, the diploid number and chromosome shape were assessed. Guerra [23] was followed in the determination of chromosomal morphology. The 18S rDNA Clusters in the Passeriformes and Gubernatrix cristata Species

To ascertain the location of ribosomal RNA gene clusters in G. cristata, biotin-labeled 18S rDNA probes were employed. Using the primers NS1 5′-GTA GTC ATA TGC TTG TCT C-3′ and NS8 5′-TCC GCA GGT TCA CCT ACG GA-3′ and nuclear DNA of Ocyurus chrysurus (Perciformes: Lutjanidae) [24], the ribosomal fragments were amplified by PCR. They were then labeled via nick translation (Roche, Mannheim, Germany) and detected using streptavidin–Cy3 in accordance with the manufacturer's instructions. Daniels and Delany were followed by hybridization, stringency washes, and detection [25]. A Zeiss Axioplan 2 fluorescent microscope and AxioVision 4.8 software (Zeiss, Jena, Germany) were used to evaluate the data.

We looked for papers that had addressed this issue in the National Center for Biotechnology Information (NCBI) database in order to compare the rDNA distribution throughout Passeriformes. The terms "18S rDNA in Passeriformes" and "FISH with 18S rDNA probes in Passeriformes" were utilized. The TimeTree database (http://www.timetree.org, accessed on 8 September 2021) provided a phylogenetic tree of the Passeri-formes species in relation to the 18S rDNA FISH results [26].

FINDINGS

G. cristata's Karyotype

The G. cristata individual under study had 78 chromosomes, comprising 27 pairs of microchromosomes and 12 pairs of macrochromosomes, including the Z chromosome (Figure 1). Submetacentric couples were the first six. It was believed that the remaining autosomes were telocentric. Z was a submetacentric chromosome.

The 18S rDNA Distribution in G. cristata According to FISH studies using 18S rDNA probes, these sequences are distributed over two pairs of microchromosomes in G. cristata.

Comparisions of 18S rDNA Distribution among Passeriformes Species

Our searches in the NCBI database resulted in a total of 10 papers, corresponding to 27 Passeriformes species in which the 18S rDNA distribution had been determined, including the present study (Table 1). The most frequent number of chromosomes with 18S rDNA clusters was two microchromosomes, however, some species showed a higher number of chromosomes, such as four and six microchromosomes. Only four species from the Thraupidae family have had the 18S rDNA clusters characterized: Saltator aurantiirostris, Tachyphonus coronatus, and Coryphospingus cucullatus with two microchromosomes with these sequences, and G. cristata with four microchromosomes. To better illustrate the 18S rDNA distribution among Passeriformes species, the numbers of chromosomes with these sequences were plotted in a phylogenetic tree.

DISCUSSION

With the recent advent of increasingly cost-effective high-throughput sequencing, most assembled genomes often lack a basic physical map or even information about chromosome numbers and morphology [36], especially in birds, due to the high number of microchromosomes [4], the high GC

content [5], and the presence of tandem repeats [6]. A direct information link between the assembled genomes and the standard karyotype of the target species is not always provided. Hence, cytogenetic analyses are an important method of understanding the connections between the DNA and chromosomal structure.

In this study, we present the first karyotype description of the endangered species yellow cardinal (Gubernatrix cristata). The karyotype of G. cristata is composed of 78 chromosomes, which is a typical avian karyotype, since approximately 61% of the total number of species karyotyped showed a diploid number between 76 and 82 [1]. This is also a typical karyotype in Passeriformes members [1,28–35].

Regarding the chromosomal morphology variation in Thraupidae karyotypes, the first four pairs generally seem to vary frequently between metacentric, submetacentric, and acrocentric [35,37]. These changes may indicate intrachromosomal rearrangements, such as pericentric inversions, considering that the sizes of these chromosomes are highly conserved in these birds. In fact, previous studies have indicated that this type of re- arrangement is frequent among Passeriformes, both with in situ [29–35] and in silico experiments [38,39].

Usually, most of the avian species have the 18S rDNA clusters in one pair of mi- crochromosomes, including in the basal species (Paleognathae) [27,40]. Therefore, this state can be considered a plesiomorphic (ancestral) condition. However, it is possible to observe apomorphic states in some species, as the 18S clusters are present in a higher number of microchromosome pairs. In addition, in other cases, these clusters can be found in macrochromosomes [27]. In G. cristata, the 18S rDNA clusters were found in
four microchromosomes. Probably, the extra clusters in G. cristata resulted from duplication of rDNA sites and redistribution via translocation [41], since the increase in the number of chromosome pairs bearing 18S rDNA is not related to a high diploid number (Table 1, Figure 3). Interestingly, previous studies have demonstrated that four Thraupidae members (Saltator similis, Saltator aurantiirostris, Tachyphonus coronatus, and Coryphospingus cucullatus) share the ancestral state [27,35].

Hence, we propose that the common ancestor of Thraupidae members had the ancestral condition, and duplication and translocation events increased the number of ribosomal clusters in G. cristata. Future studies are necessary to investigate the 18S rDNA state in other Thraupidae members. Moreover, most of the Passeriformes families that have been investigated with ribosomal probes also have the ancestral state of the 18S rDNA cluster (Table 1), indicating that the common ancestor of Passeriformes had the ancestral state, while duplication and translocations of these sequences occurred independently in some lineages

In conclusion, we demonstrated that G. cristata has a typical avian diploid number and an apomorphic condition of 18S rDNA clusters. Furthermore, we proposed that chromosomal rearrangements, such as duplication and translocation, were the main mechanisms responsible for redistribution of the clusters of 18S in G. cristata. Our data represent a starting point for understanding the genome organization and evolution of this endangered species.

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