




Article

Karyotype Organization of the Endangered Species Yellow Cardinal (*Gubernatrix cristata*)

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Abstract: Karyotypic analyses have several applications in studies of chromosome organization, evolution, and cytotaxonomy. They are also essential to genome assembly projects. Here, we present for the first time the karyotype description of the endangered species yellow cardinal, *Gubernatrix cristata* (Passeriformes, Thraupidae), using conventional staining with Giemsa and 18S rDNA probes. This species has 78 chromosomes, with 12 pairs of macrochromosomes and 27 microchromosome pairs. The 18S rDNA clusters were found in four microchromosomes. Our results revealed that *G. cristata* has a typical avian karyotype (approximately 80 chromosomes). However, *G. cristata* has an apomorphic state in relation to the 18S rDNA distribution since the ancestral condition corresponds to only two microchromosomes with these sequences. Probably, duplications and translocations were responsible for increasing the number of 18S rDNA clusters in *G. cristata*. The results were compared and discussed with respect to other Thraupidae and Passeriformes members. Considering the globally threatened status of *G. cristata*, we believe that its karyotype description could be a starting point for future cytogenetics and sequencing projects.

Keywords: Thraupidae; genome; avian chromosomes; rDNA



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1. Introduction

The field of Avian cytogenetics is still new. To date, only 9.83% of bird species have had their karyotypes described [1]. Although Passeriformes represent the order with the largest number of species karyotyped (460), this number represents only 7% of the species from the order [1], indicating that the chromosome organization and evolution in this group remain largely unknown. Considering the Thraupidae family, 11.8% of approximately 380 species have had their karyotypes described [1].

Karyotypic analyses have been used for decades in studies of chromosome organization, evolution, and cytotaxonomy in birds [2,3]. The karyotypes are also important for confirming the number of chromosomes found during genome sequencing and assembly. For instance, the number of assembled chromosomal groups for the canary (*Serinus canaria*) using whole-genome shotgun sequencing was 35 ($2n = 70$); however, the cytogenetic analysis via conventional staining with Giemsa showed that the correct number of chromosomes is 40 ($2n = 80$) [4]. This difference is due to the large number of microchromosomes [4], the high GC content [5], and the presence of tandem repeats [6] in avian species. These findings highlight the importance of karyotype description for the correct assembling of chromosomes.

The yellow cardinal (*Gubernatrix cristata*) is a member of the Thraupidae (Passeriformes, Oscines). The species is globally threatened and qualified as Endangered due to constant population decline [7]. The main threats are habitat loss, mainly due to the conversion of native fields into agricultural areas [8–10], and trapping, especially of males, for the illegal trade of birds [10–12].

The species occurs in the Pampa Biome, in southern South America. It is strongly associated with the savanna–park-type vegetation, present in large parts of Argentina and Uruguay and a few sites in the Rio Grande do Sul State, Brazil [13–16]. *G. cristata* has always been rare throughout its distribution; currently, the records are sparse and restricted to difficult-to-access points or legally protected areas [17–19]. This is the case in Brazil, where the only population, with about 50 specimens, is found in Espinilho State Park and its surrounding areas [20,21].

Due to its threat level, the species was included in a Brazilian national action plan aimed at the conservation of endangered passerines of the Campos Sulinos in Brazil. This action plan has, among its goals, the surveying of the populations and genetic data of the birds and the application of the results to improve proposals for management and conservation measures in the region [20].

There are no previous cytogenetic studies of *G. cristata*. Hence, in this study, we have performed a karyotype description including the distribution of 18S rDNA clusters in this species. Our aim was to provide cytogenetic information on this endangered species, which may be useful for future studies, especially genome assembly. In addition, we have compared our results with those of other Passeriformes species, especially Thraupidae members.

2. Materials and Methods

2.1. Animal

One male individual of *G. cristata* was used in this study. The animal was maintained in the Animal Screening Center (Centro de Triagem de Animais Silvestres) of Porto Alegre, Rio Grande do Sul State, in 2014. The animal was sent to the Center by the “Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais”, having been captured during inspection activities carried out by the agency.

2.2. Cell Culture and Karyotype Description

A cell culture was established from feather pulp from the male individual of *G. cristata*, following [22]. Briefly, the feather pulp was dissociated mechanically and with type IV collagenase for one hour. The cell suspension obtained was cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 15% of fetal bovine serum, 2% of penicillin streptomycin, and 1% of L-glutamine at 37 °C. Metaphase chromosome spreads were obtained after colcemid treatment (1 h), hypotonic solution (0.075 M KCl, 15 min), and fixation with 3:1 methanol/acetic acid. The diploid number and chromosome morphology were determined in at least 20 metaphase plates stained with Giemsa (10% in 0.07 M phosphate buffer, at pH 6.8). The chromosomal morphology determination followed Guerra [23].

2.3. The 18S rDNA Clusters in *Gubernatrix cristata* and in Passeriformes Species

Biotin-labeled 18S rDNA probes were used to determine the distribution of ribosomal RNA gene clusters in *G. cristata*. The ribosomal fragments were amplified by PCR using 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], labeled via nick translation (Roche, Mannheim, Germany) and detected with streptavidin–Cy3, following the manufacturer’s instructions. Hybridization, stringency washes, and detection followed Daniels and Delany [25]. The results were analyzed using a Zeiss Axioplan 2 fluorescent microscope and AxioVision 4.8 software (Zeiss, Jena, Germany).

To perform a comparison of the rDNA distribution among Passeriformes, we searched in the National Center for Biotechnology Information (NCBI) database for studies that have

addressed this theme. The following keywords were used: “18S rDNA in Passeriformes” and “FISH with 18S rDNA probes in Passeriformes”. A phylogenetic tree of the Passeriformes species with respect to the 18S rDNA FISH results was sourced from the TimeTree database (<http://www.timetree.org>, accessed on 8 September 2021) [26].

3. Results

3.1. The Karyotype of *G. cristata*

The *G. cristata* individual analyzed here showed 78 chromosomes, with 12 pairs of macrochromosomes including the Z chromosome, and 27 microchromosome pairs (Figure 1). The first 6 pairs were submetacentric. The remaining autosomes were considered as telocentric. The Z chromosome was submetacentric.

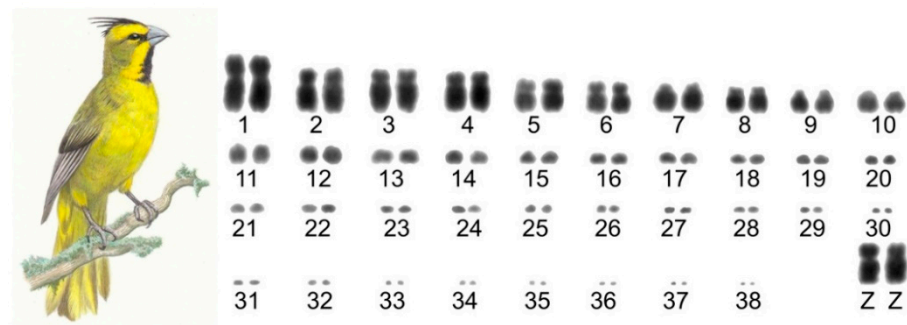


Figure 1. Complete karyotype of a male *Gubernatrix cristata* ($2n = 78$), with conventional Giemsa staining.

3.2. The 18S rDNA Distribution in *G. cristata*

FISH experiments with 18S rDNA probes indicated that these sequences are distributed in four microchromosomes (two pairs) in *G. cristata* (Figure 2).

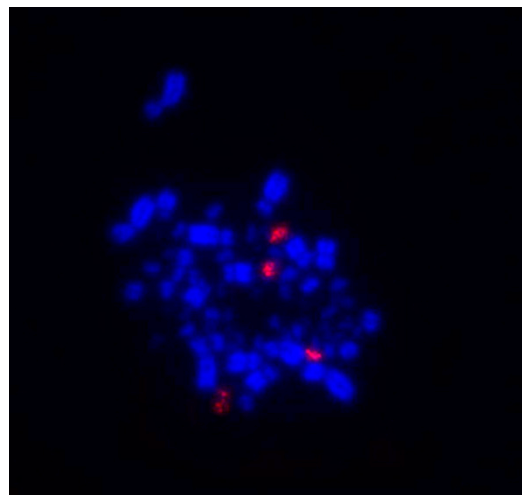


Figure 2. 18S rDNA distribution in *Gubernatrix cristata*, found in four microchromosomes (two pairs).

3.3. Comparisons 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 (Figure 3).

Table 1. List of Passeriformes species with 18S rDNA chromosome mapping.

Species	Family	Suborder	No ¹	Diploid Number	Reference
<i>Syndactyla rufosuperciliata</i>	Furnariidae	Suboscines	2 micros	82	[27]
<i>Cranioleuca obsoleta</i>	Furnariidae	Suboscines	2 micros	82	[27]
<i>Furnarius rufus</i>	Furnariidae	Suboscines	2 micros	82	[27]
<i>Synallaxis albescens</i>	Furnariidae	Suboscines	2 micros	82	[27]
<i>Anumbius annumbi</i>	Furnariidae	Suboscines	2 micros	82	[27]
<i>Dendrocolaptes platyrostris</i>	Furnariidae	Suboscines	2 macros	82	[27]
<i>Glyphorhynchus spirurus</i>	Furnariidae	Suboscines	2 macros	80	[28]
<i>Schiffornis virescens</i>	Tityridae	Suboscines	2 micros	82	[27]
<i>Myiarchus ferox</i>	Tyrannidae	Suboscines	2 micros	76	[27]
<i>Pitangus sulphuratus</i>	Tyrannidae	Suboscines	2 micros	80	[29]
<i>Satrapa icterophrys</i>	Tyrannidae	Suboscines	2 micros	82	[29]
<i>Serpophaga subcristata</i>	Tyrannidae	Suboscines	4 micros	82	[29]
<i>Elaenia spectabilis</i>	Tyrannidae	Suboscines	4 micros	80	[30]
<i>Conopophaga lineata</i>	Conopophagidae	Suboscines	2 micros	78	[31]
<i>Taeniopygia guttata</i>	Estrildidae	Oscines	2 micros	80	[32]
<i>Basileuterus culicivorus</i>	Parulidae	Oscines	2 micros	80	[27]
<i>Serinus canaria</i>	Fringillidae	Oscines	4 micros	80	[32]
<i>Agelaioides badius</i>	Icteridae	Oscines	4 micros	80	[27]
<i>Molothrus bonariensis</i>	Icteridae	Oscines	2 micros	80	[27]
<i>Turdus rufiventris</i>	Turdidae	Oscines	6 micros	78	[33]
<i>Turdus albicollis</i>	Turdidae	Oscines	4 micros	78	[33]
<i>Zonotrichia capensis</i>	Passerellidae	Oscines	2 micros	80	[34]
<i>Saltator similis</i>	Thraupidae	Oscines	2 micros	80	[35]
<i>Saltator aurantirostris</i>	Thraupidae	Oscines	2 micros	80	[35]
<i>Tachyphonus coronatus</i>	Thraupidae	Oscines	2 micros	80	[27]
<i>Coryphospingus cucullatus</i>	Thraupidae	Oscines	2 micros	80	[27]
<i>Gubernatrix cristata</i>	Thraupidae	Oscines	4 micros	78	Present study

¹ Number of chromosomes with 18/28S rDNA clusters.

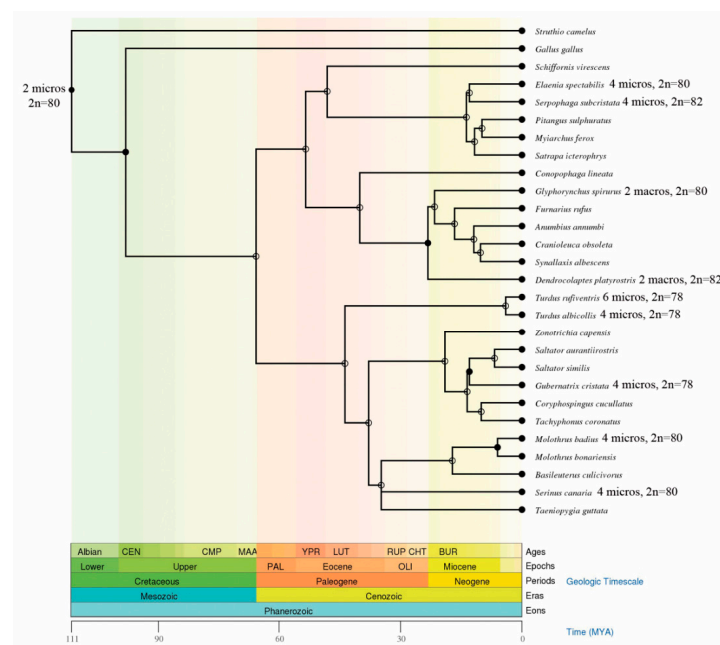


Figure 3. Phylogeny of the Passeriformes species with 18S rDNA from FISH results. Most of the species have the ancestral state (two microchromosomes). Species with apomorphic condition are indicated in the respective branches. The phylogenetic tree was sourced from the TimeTree database (<http://www.timetree.org>, accessed on 8 September 2021) [26].

4. 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 rearrangement 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 microchromosomes, 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 (Table 1).

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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Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the article.

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Conflicts of Interest: The authors declare no conflict of interest and the funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript or in the decision to publish the results.

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