*proceedings*

MDPI

*Proceedings*

# Revising the chromosome-specific probes of white hawk (*Leucopternis albicollis*) †

**Ivanete Furo [1,*], Rafael Kretschmer [2], Jorge Pereira [3], Darren Griffin [4], Rebecca O'Connor [4], Patricia O'Brien [5], Malcolm Ferguson-Smith [5] and Edivaldo de Oliveira [6,7]**

[1] Laboratório de Reprodução Animal, LABRAC, Universidade Federal Rural da Amazônia, UFRA, Parauapebas, PA, Brazil
[2] Programa de Pós-graduação em Genética e Biologia Molecular, PPGBM, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; rafa.kretschmer@hotmail.com
[3] Animal and Veterinary Research Centre (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; Jorgecpereira599@gmail.com
[4] School of Biosciences, University of Kent, Canterbury CT2 7NJ, UK; D.K.Griffin@kent.ac.uk (D.G.); R.O'Connor@kent.ac.uk (R.O.)
[5] Cambridge Resource Centre for Comparative Genomics, University of Cambridge Department of Veterinary Medicine, Cambridge, UK; allsorter@gmail.com (P.O.); maf12@cam.ac.uk (M.F.-S.)
[6] Laboratório de Cultura de Tecidos e Citogenética, SAMAM, Instituto Evandro Chagas, Ananindeua, PA, Brazil; ehco@ufpa.br
[7] Instituto de Ciências Exatas e Naturais, Universidade Federal do Pará, Belém, PA, Brazil
* Correspondence: ivanetefuro100@gmail.com
† Presented at the 1st International Electronic Conference on Genes: Theoretical and Applied Genomics, 2–30 November 2020; Available online: https://iecge.sciforum.net/.

Received: date; Accepted: date; Published: date

**Abstract:** *Leucopternis albicollis* is a diurnal bird of prey with extensive karyotype reorganization. Chromosome-specific probes from this species have been used successfully to detect intrachromosomal rearrangements in different species of bird since 2010. However some gaps were detected in this first set of probes. Here, we have obatined a new set of whole chromosome probes in order to improve the previous one; also we have performed experiments using bacterial artificial chromosome (BAC) from chicken microchromosomes. Our results demonstrated that the microchromosomes were involved in fusion events. In addition, a new nomenclature has been proposed for the new set of probes and some inaccurate data were corrected.

**Keywords:** flow karyotype; microchromosomes; fusions; nomenclature

## 1. Introduction

Whole chromosome probes have provided the opportunity to analyze the chromosome homology, organization and evolution of different taxa with more accuracy than methods based on conventional banding techniques. The first avian set of chromosome paints was obtained from *Gallus gallus* (chicken-GGA) and has become a powerful tool to detect chromosome rearrangements and chromosome signatures between different species, facilitating the proposal of phylogenetic inferences [1,2]. However, considering that most species of birds have retained karyotypes similar to *G. gallus*, with maintenance of most syntenic groups, the application of *GGA* paints is not able to detect intrachromosomal rearrangements, such as inversions, apart the few cases in which chromosome fusions occurred previously to the inversion events, such as in some Psitaciformes [3].

An alternative approach to the limitations of *G. gallus* probes was the use of whole chromosome paints obtained from a species in which syntenic groups have been fissioned or reshuffled, such as the white hawk (*Leucopternis albicollis*) [4]. Despite having a diploid number of 2n = 66, the ancestral

macrochromosomes 1-3 (GGA1-3) and 5 (GGA5) have been fissioned in *L. albicollis* [4]. For instance, from the sorted chromosomes of *L. albicollis*, five different pairs were assingned to GGA1. Hence, the use of these paintings allowed the detection of intrachromosomal rearrangements in species with the ancestral chromosome 1 conserved as an entire chromosome, such as in Columbiformes [5], or in species with fissions in this chromosome, such as in Passeriformes [6]. However, the fact that the five previously chromosomes of *L. albicollis* identified as homologous to GGA1 did not cover the entire chromosome 1 of Columbiformes, species raised the suspicious that, at least, one more chromosome of *L. albicollis* should be assigned to GGA1 [5]. Similar results were observed in Opisthocomiformes and Cuculiformes [7,8]. Hence, the aim of this study was to generate a new set of chromosome paints of *L. albicollis* in order to obtain a better coverage of GGA chromosomes. In addition, we performed fluorescence in situ hybridization (ISH) using bacterial artificial chromosomes (BACs) of *G. gallus* microchromosomes in order to demonstrate which microchromosome pairs were involved in fusions, which played an important role in the karyotype evolution of *L. albicollis*.

## 2. Material and Methods

### *2.1. Ethics Committee*

The experiments were performed followed ethical protocols approved by the ethics committee (CEUA—Federal University of Para) no. 170/2013.

### *2.2. Flow Sorting*

Flow sorting was performed according to Nie et al. [10]. Chromosome preparations were obtained from a fibroblast cell line of a female *Leucopternis albicollis*. Chromosomes were stained with chromomycin A3 (40 μg/mL, Sigma) and Hoechst 33,258 (2 μg/mL, Sigma). Sorting was carried out using a dual-laser cell sorter (MoFlo, Beckman Coulter). The primary sorted chromosome material was amplified by degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) [11] and then resulting products were labeled with biotin-16-dUTP during secondary DOP-PCR amplification. The identity of probes was validated by same-species chromosome painting.

### *2.3. Chicken BAC Clones and Hybridization In Situ*

Generation, labeling and experiments using bacterial artificial chromosomes (BACs) followed O'Connor et al. [12]. BACs were selected from the genome library of *Gallus gallus* (GGA). The slides were analyzed with an Olympus BX-61 epifluorescence microscope equipped with a cooled CCD camera and appropriate filters. Images were captured using SmartCapture3 (Digital scientific UK).

## 3. Results

### *3.1. Flow Karyotype*

The 66 chromosomes of *L. albicollis* were resolved into 33 peaks by flow cytometry (Figure 1). The chromosomes in each peak of the flow karyotype were identified on *L. albicollis* metaphases using FISH with labeled peak-specific DNA (Figure 2)

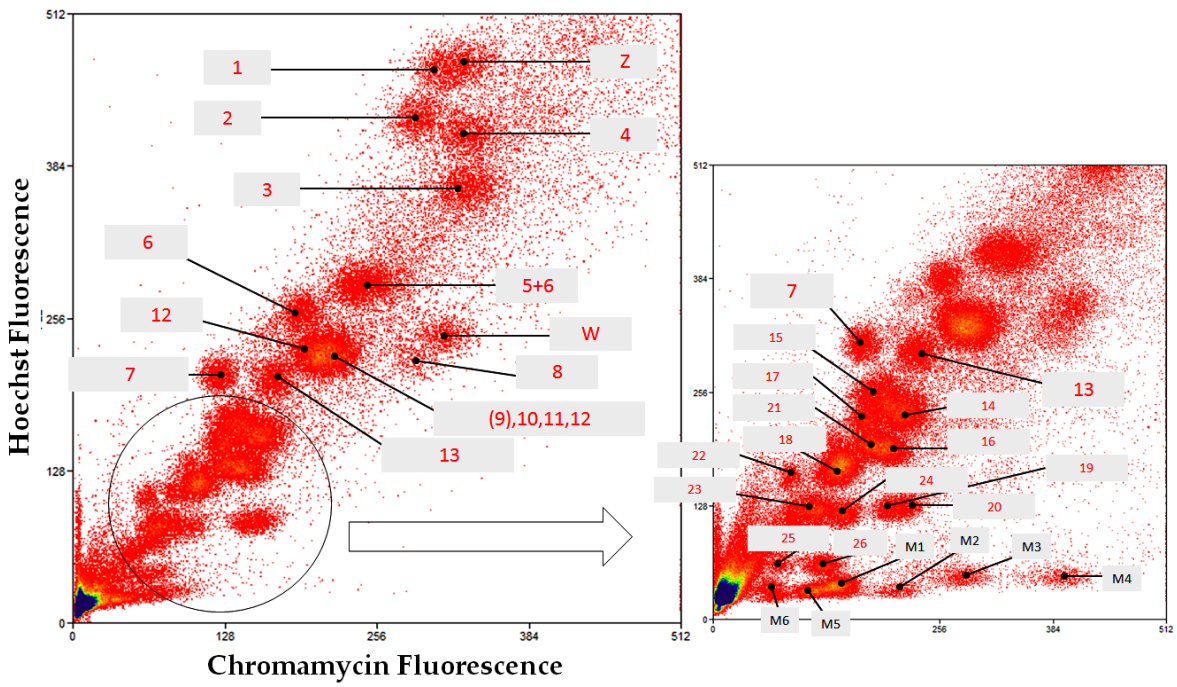


**Figure 1.** New flow karyotype of *L. albicollis*. Legend: M = Microchromosomes.

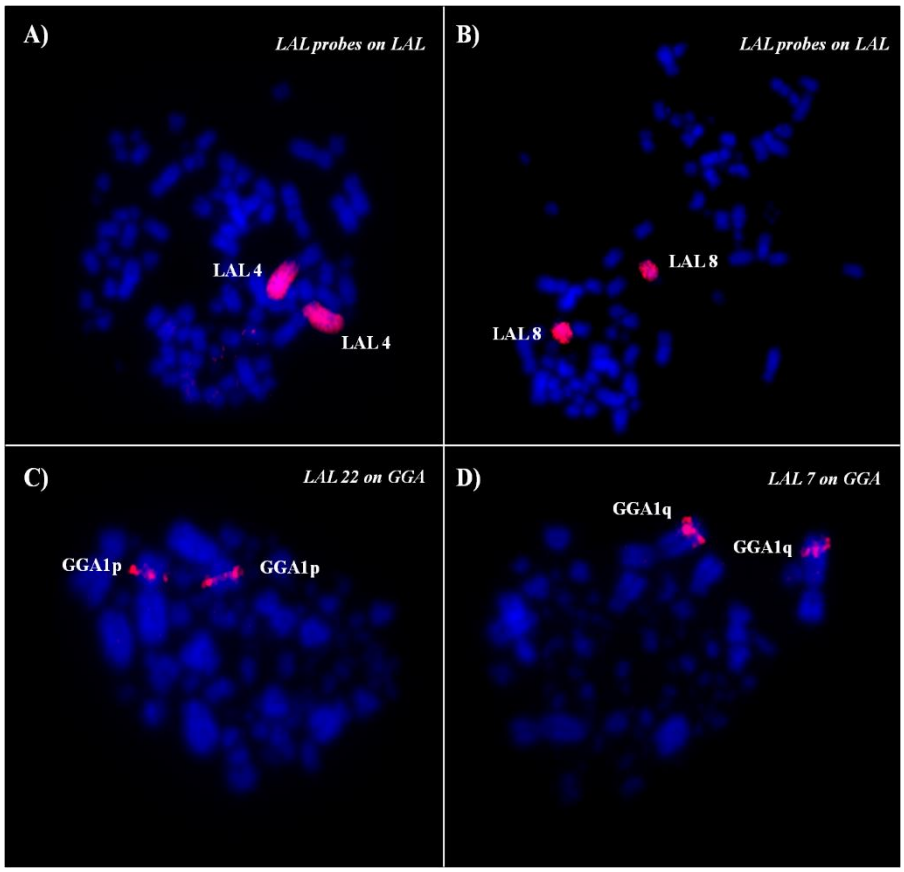


**Figure 2.** Representative Same-species FISH experiments using *L. albicollis* (LAL) probes: LAL 4 (**A**), LAL8 (**B**) and Cross-species LAL probes on *G.gallus* metaphases (**C**,**D**).

### *3.2. BAC-FISH Mapped onto L. albicollis Chromosomes*

BAC probes corresponding to GGA chromosome pairs 17–28, except pair 20 (which did not produce reproducible results) demonstrated that all the microchromosomes were involved in fusion events on karyotype of *L. albicollis*.

### *3.3. Chromosome Painting between L. albicollis and Gallus gallus*

Reciprocal chromosome painting between *L. albicollis* and *G. gallus* established chromosome homologies between these species which are summarized in the Figure 3. The GGA1 was formally represented by five different pairs of *L. albicollis* (Figure 3A) [4], however, in our analysis we found two extra small pairs, summing seven chromosomes of white hawk covering GGA1 (Figure 3B).

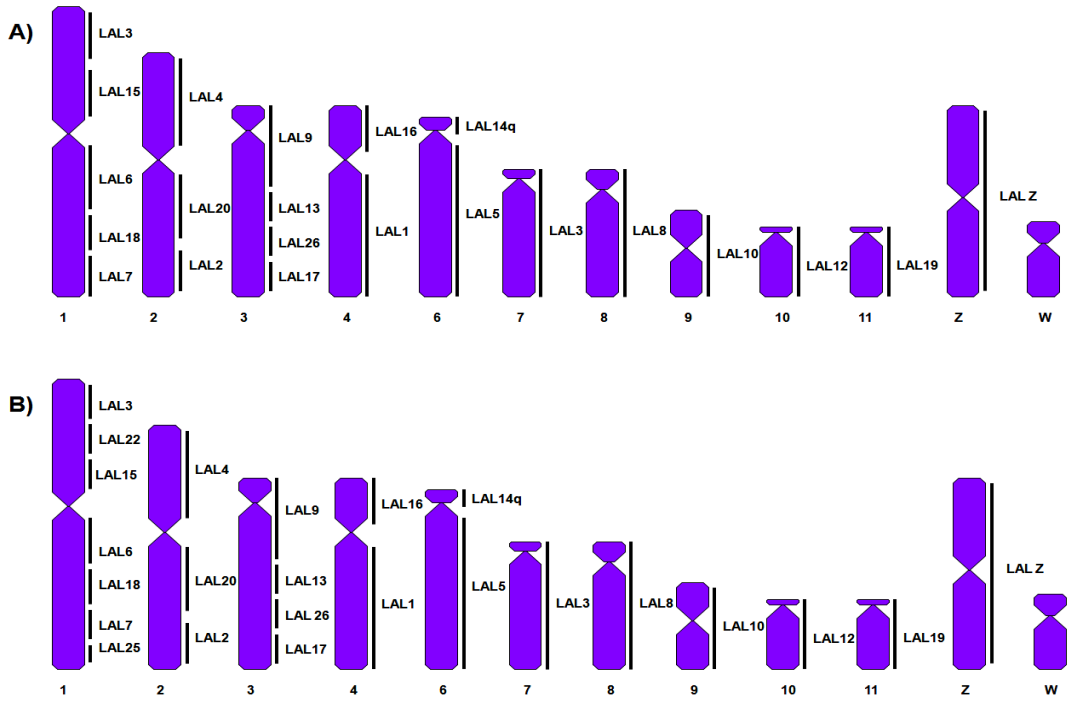


**Figure 3.** Homology map between chicken macrochromosomes and white hawk paints. (**A**) The homology described by de Oliveira et al. [4] (**B**) The new nomenclature has been proposed for the new set of probes from white hawk.

## 3. Discussion

In this study we provided a new set of whole chromosome probes from *L. albicollis* obtained by flow cytometry, which contributed to the improving the previously set one [4]. Specifically, we provided here the isolation and identification of some chromosomes which were missing in the first set. For instance, it has been proposed that five chromosomes of *L. albicollis* were homologous to chromosome 1 of *Gallus gallus* [1], however, here we confirmed that are seven pairs of *L. albicollis* homologous to GGA1. These two small chromosomes, identified as pairs 22 and 26 (Figure 3), were not sorted as individual paints in the previous description, making it difficult to assign them in the homology map.

*L. albicollis* probes have been used in cytogenetics studies of birds since 2010, and intrachromosomal rearrangements were proposed in the chromosome homologous do GGA1 in some species, especially in Passeriformes and Columbiformes [5,6]. Although these studies have applied only five whole chromosome pairs of *L. albicollis* as homologous to GGA1, the occurrence of intrachromosomal rearrangements is still valid, however, the use of the two additional chromosomes identified herein as homologous to GGA1 (LAL 22 and LAL 25) (Figures 2 and 3) are involved in the rearrangements proposed or are conserved as an entire segments in species of Passeriformes and Columbiformes. Hence, intrachromosomal rearrangements proposed in Passeriformes and

Columbiformes [5,6], may represent an even more complex series of inversions than initially proposed.

Considering that *L. albicollis* has 66 chromosomes, despite the occurrence of innumerous fission events in macrochromosomes, and a few pair of microchromosomes, raised the idea that fusions involving these small elements were important in the karyotypical evolution of this species [4]. Hence, we confirmed this assumption and identifyied chromosome fusions involving GGA microchromosomes 17-28 (except 20, see material and methods). Chromosome fusions involving microchromosomes have been previously detect only in Falconiformes and Psitaciformes species [12].

As a conclusion, our results have improved the set of *L. albicollis* probes for comparative chromosome painting in birds and identified the homologous to GGA microchromosomes involved in fusions in the karyotype of the *L. albicollis*, by the use of BAC derived probes.

**Author Contributions:** Conceptualization, I.F. and E.d.O.; methodology, I.F. and J.P.; validation, I.F. and E.d.O.; formal analysis, I.F., investigation, I.F., J.P.; resources, E.d.O.; M.F.-S., D.G., and R.O.; data curation, I.F., R.K. and E.d.O.; writing—original draft preparation, I.F., R.K. and E.d.O.; writing—review and editing, I.F., R.K., E.d.O., P.O., M.F.-S., D.G. and R.O.; supervision and project administration, E.d.O., M.F.-S.; funding acquisition, M.F.-S. and E.d.O. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was partially funded by a grant to EHCO from CNPq (307382/2019-2) and to MAFS from the Wellcome Trust in support of the Cambridge Resource Centre for Comparative Genomics and by the Biotechnology and Biological Sciences Research Council (BB/K008161/1) to the University of Kent, and by the project UIDB/CVT/00772/2020 funded by the Fundação para a Ciência e Tecnologia (FCT).

**Acknowledgments:** We are very grateful to CNPq (309699/2015-0) and CAPES for technical and financial support.

**Conflicts of Interest:** The authors declare that they have no conflict of interest

## References

1. Griffin, D.K.; Robertson, L.B.W.; Tempest, H.G.; Skinner, B.M. The evolution of the avian genome as revealed by comparative molecular cytogenetic. *Cytogenet. Genome Res.* **2007**, *117*, 64–77.
2. Kretschmer, R.; Ferguson-Smith, M.A.; de Oliveira, E.H.C. Karyotype evolution in birds: From conventional staining to chromosome painting. *Genes* **2018**, *9*, 181.
3. Nanda, I.; Karl, E.; Griffin, D.K.; Schartl, M.; Schmid, M. Chromosome repatterning in three representative parrots (Psittaciformes) inferred from comparative chromosome painting. *Cytogenet. Genome Res.* **2007**, *117*, 43–53.
4. De Oliveira, E.H.C.; Tagliarini, M.M.; Rissino, J.D.; Pieczarka, J.C.; Nagamachi, C.Y.; O'Brien, P.C.; Ferguson-Smith, M.A. Reciprocal chromosome painting between white hawk (*Leucopternis albicollis*) and chicken reveals extensive fusions and fissions during karyotype evolution of Accipitridae (Aves, Falconiformes). *Chromosome Res.* **2010**, *18*, 349–355.
5. Kretschmer, R.; de Oliveira Furo, I.; Gunski, R.J.; del Valle Garnero, A.; Pereira, J.C.; O'Brien, P.C.; Ferguson-Smith, M.A.; de Oliveira, E.H.C.; de Freitas, T.R.O. Comparative chromosome painting in Columbidae (Columbiformes) reinforces divergence in Passerea and Columbea. *Chromosome Res.* **2018**, *26*, 211–223.
6. Kretschmer, R.; Gunski, R.J.; Garnero, A.D.V.; de Oliveira Furo, I.; O'Brien, P.C.; Ferguson-Smith, M.A.; de Oliveira, E.H.C. Molecular cytogenetic characterization of multiple intrachromosomal rearrangements in two representatives of the genus *Turdus* (Turdidae, Passeriformes). *PLoS ONE* **2014**, *9*, e103338.
7. Dos Santos, M.S.; Furo, I.O.; Tagliarini, M.M.; Kretschmer, R.; O'Brien, P.C.M.; Ferguson-Smith, M.A.; de Oliveira, E.H.C. The Karyotype of the Hoatzin (*Opisthocomus hoazin*)—A Phylogenetic Enigma of the Neornithes. *Cytogenet. Genome Res.* **2018**, *156*, 158–164.
8. dos Santos, M.D.S.; Kretschmer, R.; Furo, I.D.O.; Gunski, R.J.; del Valle Garnero, A.; Valeri, M.P.; O'Brien, P.C.; Ferguson-Smith, M.A.; de Oliveira, E.H.C. Chromosomal evolution and phylogenetic considerations in cuckoos (Aves, Cuculiformes, Cuculidae). *PLoS ONE* **2020**, *15*, e0232509.
9. Furo, I.D.O.; Kretschmer, R.; O'Brien, P.C.; Pereira, J.C.; Garnero, A.D.V.; Gunski, R.J.; O'Connor, R.E.; Griffin, D.K.; Gomes, A.J.B.; Ferguson-Smith, M.A.; et al. Phylogenetic Analysis and chromosome evolution

in Neotropical Psittacidae with emphasis on a species with high karyotypic reorganization (*Myiopsitta monachus*). *Front. Genet* **2020**, *11*, 721.
10. Nie, W.; O'Brien, P.C.; Fu, B.; Wang, J.; Su, W.; He, K.; Bed'Hom, B.; Volobouev, V.; Ferguson-Smith, M.A.; Dobigny, G.; et al. Multidirectional chromosome painting substantiates the occurrence of extensive genomic reshuffling within Accipitriformes. *BMC Evol. Biol.* **2015**, *15*, 205.
11. Telenius, H.; Ponder, B.A.; Tunnacliffe, A.; Pelmear, A.H.; Carter, N.P.; Ferguson-Smith, M.A.; Behmel, A.; Nordenskjöld, M.; Pfragner, R. Cytogenetic analysis by chromosome painting using DOP-PCR amplified flow-sorted chromosomes. *Genes Chromosomes Cancer* **1992**, *4*, 257–263.
12. O'Connor, R.E.; Kiazim, L.; Skinner, B.; Fonseka, G.; Joseph, S.; Jennings, R.; Larkin, D.M.; Griffin, D.K. Patterns of microchromosome organization remain highly conserved throughout avian evolution. *Chromosoma* **2019**, *128*, 21–29.