Extensive Homology of Chicken Macrochromosomes in the Karyotypes of *Trachemys scripta elegans* and *Crocodylus niloticus* Revealed by Chromosome Painting despite Long Divergence Times

F. Kasai^a P.C.M. O'Brien^a S. Martin^b M.A. Ferguson-Smith^a

^aDepartment of Veterinary Medicine, University of Cambridge, Cambridge, UK; ^bLa Ferme aux Crocodiles, Pierrelatte, France

Key Words

Chicken • Chromosome rearrangement • Genome conservation • Reptile

Abstract

We report extensive chromosome homology revealed by chromosome painting between chicken (*Gallus gallus domesticus*, GGA, 2n = 78) macrochromosomes (representing 70% of the chicken genome) and the chromosomes of a turtle, the red-eared slider (*Trachemys scripta elegans*, TSC, 2n = 50), and the Nile crocodile (*Crocodylus niloticus*, CNI, 2n = 32). Our data show that GGA1–8 arms seem to be conserved in the arms of TSC chromosomes, GGA1–2 arms are separated and homologous to CNI1p, 3q, 4q and 5q. In addition to GGAZ homologues in our previous study, large-scale GGA autosome synteny have been conserved in turtle and crocodile despite hundreds of millions of years divergence time. Based on phylogenetic hypotheses that crocodiles diverged after the divergence of birds and turtles, our results in CNI suggest that GGA1–2 and TSC1–2 represent the ancestral state and that chromosome fissions followed by fusions have been the mechanisms responsible for the reduction of chromosome number in crocodiles.

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Cross-species chromosome painting has revealed detailed patterns of chromosome homology between representative species of eutherian mammalian orders. This enables the identification of chromosome rearrangements occurring during evolution and allows the construction of putative ancestral karyotypes [Ferguson-Smith and Trifonov, 2007]. Compared to the numerous rearrangements observed in mammalian chromosomes, painting between *Gallus gallus domesticus* macrochromosomes (GGA1–10) and the chromosomes of other birds shows extensive conservation with few rearrangements [Nanda et al., 2011]. However, in our experience chromosome painting cannot demonstrate the conservation between eutherians, marsupials and monotremes shown by genome sequencing, nor the conservation of any of these taxa and birds. Thus, our previous painting study gave unexpected results when it revealed that the chromosome region homologous to the chicken Z chromosome could be identified in representatives of squamates, crocodile and turtle species [Pokorná et al., 2011]. Although cDNA mapping shows homology of 26 loci between chicken 1–5 and the Chinese soft-shelled turtle (*Pelodiscus sinensis*, 2n = 66) 1–5 chromosomes [Matsuda et al., 2005], to date large-scale homology to chicken autosomes has not been shown in turtle and crocodile.

Small diploid numbers are a feature of crocodiles compared to birds and turtles [Cohen and Gans, 1970] and it

Fig. 1. The DAPI-stained karyotype of the red-eared slider (TSC, 2n = 50; **a**) and Giemsa-stained karyotype of the Nile crocodile (CNI, 2n = 32; **b**) with homology of chicken to the right of each chromosome.

has been speculated that numerous chromosome fusions leading to the low chromosome number must have occurred after their divergence from other reptiles. However, the precise mechanism of rearrangements is unknown.

Both cytogenetic studies and sequence data have been used to determine cross-species chromosome homology, but in reptiles the genome sequence database is limited and the complete analysis of chromosomes and genomes is still lacking. We now extend our comparative chromosome painting study to investigate the homology of chicken chromosomes 1–8 to the chromosomes of a turtle and a crocodile. Our results indicate extensive large chromosome synteny between chicken, turtle and crocodile, and show that the rearrangements occurring in crocodile karyotype evolution must have resulted from fissions followed by fusions.

Materials and Methods

The red-eared slider (*Trachemys scripta elegans*, TSC) and Nile crocodile (*Crocodylus niloticus*, CNI) cells were grown from embryonic tissues obtained from La Ferme aux Crocodiles. Chromosome-specific DNA was made from chromosomes sorted by flow

Table 1. Chromosome correspondence between chicken, the red-eared slider and the Nile crocodile revealed by FISH using GGA, TSC and CNI probes

Whole chromosome probe	Chromosomes hybridised		
	GGA	TSC	CNI
GGA1		1	1p+3q
GGA2		2	4q+5
GGA3		3	1q
GGA4		5+7p	2p
GGA5		4q	2q
GGA6		7q	
GGA7		11	3p
GGA8		8q	4p
GGAZ		6 ^a	6 ^a
TSC1	1		1p+3q
TSC2	2		4q+5
TSC3	3		1q
TSC4	5		2q
TSC5	4q		2p
TSC6	Z		6
TSC7	4p+6		
TSC11	7q		3p
CNI3	7+1p		
CNI5	2q		

^a Data reported previously by Pokorná et al. [2011].

cytometry; methods and flow karyotypes of TSC and CNI are given in Kasai et al., 2012. Metaphase slide preparations were made according to conventional protocols as described in Rens et al. [2006]. The identity of chromosome-specific DNA was verified by labelling with biotin-16-dUTP (Roche Applied Science, Penzberg, Germany) by hybridisation to respective metaphases using standard FISH protocols. Briefly, the freshly made metaphase slides were denatured in 0.1 M sodium hydroxide for 20–25 s and dehydrated through an ethanol series. Post-hybridisation washing for cross-species experiments was performed in 50% formamide/2× SSC at 39°C for 10 min. Hybridisation signals were detected by Cy3-avidin (GE Healthcare, Little Chalfont, UK) and slides were counterstained by Vectashield with DAPI (Vector Laboratories, Burlingame, Calif., USA). Images were taken by a cooled CCD camera mounted on a Leica DMRXA microscope. FISH images were analysed using the Leica CW4000 QFISH software or Adobe Photoshop CS2.

Results

The red-eared slider has 14 pairs of macrochromosomes and 22 indistinguishable microchromosomes (fig. 1a) and the Nile crocodile has 32 chromosomes (fig. 1b). Paint probes from GGA1–8, Z and W sex chro-

Fig. 2. Examples of chromosome painting between GGA, TSC and CNI chromosomes. GGA7 hybridised to TSC11 (a); GGA4 to TSC5 and 7p (b); GGA5 to TSC4q (c); TSC5 on GGA4q (d); TSC6 to GGAZ (e); GGA3 to CNI1q (f); TSC4 to CNI2q (g); GGA8 to CNI4p (h); TSC6 to CNI6 (i). Scale bars represent 10 μ m.

mosomes, and from TSC1–7 and 11 paints were hybridised to metaphases of *T. s. elegans* and *G. g. domesticus*, respectively, and both paint sets were hybridised to CNI metaphases. Paint probes from CNI chromosomes 3 and 5 were hybridised to GGA metaphases. The summary of the signal patterns is shown in table 1. GGA1, 2, 3, 7 and Z paints hybridised to the entire length of TSC1, 2, 3, 11 (fig. 2a) and 6, respectively. GGA4 was detected on TSC5 and 7p (fig. 2b), GGA5 on TSC4q (fig. 2c), GGA6 on TSC7q and GGA8 on TSC8q. The GGAW paint did not show specific hybridisation signals to the chromosomes of either reptilian species.

Reciprocal painting of TSC1, 2 and 3 gave the expected results without significant hybridisation to other chromosomes. TSC4 is found on GGA5, and TSC5 on GGA4q (fig. 2d). TSC6 exclusively hybridises to the entire length of GGAZ (fig. 2e), and TSC7 was detected on GGA4p and 6. GGA1 and TSC1 each paint both CNI1p and 3q. GGA2 and TSC2 each hybridise to both CNI4q and 5. GGA3, 4 and 5 hybridise to TSC3, 5 plus 7p and 4q, respectively, and were detected in CNI1q (fig. 2f), 2p (fig. 2g) and 2q, respectively. CNI3p was painted by GGA7 and TSC11, and CNI4p was painted by GGA8

(fig. 2h). Only TSC6, homologous to GGAZ, shows signals on a single pair of CNI6 (fig. 2i). However, hybridisation signals neither of GGA nor TSC probes were detected at proximal regions of CNI2q, 3p and 4p chromosome arms (fig. 3). Homology between the 3 species based on GGA and TSC painting probes is shown in the idiograms (fig. 3). Reciprocal painting also revealed that CNI3 painted both GGA1p and 7, and that CNI5 was hybridised to GGA2q, thus identifying the arms of GGA1 and 2 that were homologous to the arms of CNI1p, 3q, 4q and 5 (table 1).

Some signals from each of the above probes were seen on chicken and turtle microchromosomes. Although these could represent regions of conserved synteny on microchromosomes, or cryptic interchromosomal rearrangements, they could not be distinguished from repetitive DNA in the low stringency conditions required for cross-species hybridisation. Increase of probe concentration up to 4 and 8 times did not improve hybridisation efficiency and caused high background. Our images show the level of background without the removal of background by image processing.

Fig. 3. The phylogenetic tree between *G. g. domesticus*, *T. s. elegans* and *C. niloticus* is based on 2 hypotheses; a branch point leading to crocodile lineage is different between morphological (A) and molecular (B) studies. Idiograms show chromosome homology of GGA1–8 and Z with TSC and CNI chromosomes revealed by cross-species chromosome painting. Homologous regions are identified by colour. The uncoloured regions, TSC4p and 8p have no homology to GGA1–8 and Z. Fissions of ancestral metacentric chromosomes have occurred during crocodile evolution, resulting in the formation of metacentric chromosomes with other acrocentric chromosomes by fusion.

Discussion

In view of the failure of chromosome painting to demonstrate chromosome homology between eutherians, marsupials and monotremes, it is surprising that cross-species painting reveals extensive chromosome homology, corresponding to 70% of the chicken genome, between the much more distantly-related extant bird and reptile species whose common ancestor existed 230 million years ago [Shedlock and Edwards, 2009]. It is known that rates of nucleotide substitution are an order of magnitude slower in birds and reptiles than in mammals [Burt, 2002; Shedlock et al., 2007], and this sequence similarity may explain the success of cross-species painting between birds and reptiles. Also, there is some evidence that chicken and reptile genomes contain a higher percentage of long-conserved non-coding sequences than mammals [Janes et al., 2011], and this may be a factor which deserves further study.

In this study, we show remarkable conservation of macrochromosomes between *G. g. domesticus* and *T. s. elegans*, consistent with the comparative gene mapping data of Matsuda et al. [2005] between chicken and the Chinese soft-shelled turtle. Both studies suggest that bird and turtle macrochromosome synteny has remained intact since their divergence from a common ancestor, and that these large macrochromosomes may also be conserved between turtles. Further comparisons of GGA chromosome homology with other turtles will uncover the extent of rearrangement of the smaller chromosomes in the known variation of turtle karyotypes [Valenzuela

and Adams, 2011]. In most squamates, gene mapping [Srikulnath et al., 2009] and chromosome painting [Pokorná et al., in press] show GGA chromosomes 3, 5 and 7 associated in one of the largest metacentric chromosomes, i.e. a rearrangement distinct from any that we find in turtle and crocodile.

The phylogenetic position of turtles and crocodiles among the major groups of living birds and reptiles is uncertain. While morphological data and the fossil record suggest that *C. niloticus* belongs to the same branch as *G. g. domesticus* [Lyson et al., 2010], molecular data tend to favour *C. niloticus* as a sister group to *T. s. elegans* [Hedges and Poling, 1999; Nakatani et al., 2007; Shedlock et al., 2007]. We have been able to determine CNI1–6 chromosome homologies to both GGA and TSC macrochromosomes. Both long and short arms of GGA1 and 2 are separated and rearranged in CNI1, 3, 4 and 5. Assuming that the ancestral karyotype is closer to *G. g. domesticus* and *T. s. elegans* rather than to *C. niloticus*, these rearrangements imply that fission of metacentric chromosomes initiated fusions resulting in CNI1, 3, and 4. A further centric fusion has been observed in the Siam crocodile (*C. siamensis*, CSI, $2n = 30$), in which CNI6 is homologous to CSI3p [Kawai et al., 2007]. Thus, several rearrangements have led to the reduction in chromosome number during crocodile evolution. Despite this, synteny of GGA, TSC and CNI chromosome arms seem to be well-conserved after their divergence from their common ancestor.

Because of our early success with painting GGAs across reptile species, it was thought that this might be

due to a higher degree of conservation of the Z sex chromosome compared to autosomes, consistent with the high conservation of the X sex chromosome found across all mammalian species (Ohno's law). However, our results now demonstrate conservation of GGA large autosomes similar to that of GGAZ.

GGA microchromosome homology has not yet been studied in reptiles and cryptic or intrachromosomal rearrangements can only be revealed by gene mapping and sequencing. Such studies may provide clues that inform the phylogenetic relationships between these taxa. The extensive chromosome conservation that we report may also contribute to the similar genome sizes in chicken, turtle and crocodile [Kasai et al., 2012].

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