

Reassignment of chicken W chromosome sequences to the Z chromosome by fluorescence in situ hybridization (FISH)

R. Stiglec T. Ezaz J.A.M. Graves

Comparative Genomics Group, Research School of Biological Sciences, The Australian National University, Canberra (Australia)

Manuscript received 7 April 2006; accepted in revised form for publication by M. Schmid, 26 May 2006.

Abstract. There is much interest in the gene content of the small heterochromatic W chromosome of the chicken, on the supposition that it may contain sex-determining genes. A considerable region in the chicken genome has been assigned to the W chromosome on the basis of its repetitive sequences. Using fluorescent in situ hybridization (FISH) we localized five Bacterial Artificial Chromosomes

(BACs) onto female chicken metaphase spreads. We physically mapped these BACs to the Z chromosome. The chicken genome database, however, assigned all five BACs to the W chromosome. Our results demonstrate that the 17 genes on these BACs are Z-specific, and points to the inadequacy of assigning regions of the genome based exclusively on repetitive sequences.

Copyright © 2007 S. Karger AG, Basel

Birds subscribe to a ZZ male:ZW female system of sex determination, but it is still unknown whether the W chromosome contains female-determining genes (Smith and Sinclair, 2001, 2004). The gene content of the small, heterochromatic chicken W chromosome is therefore of considerable interest (Reed and Sinclair, 2002; Yamada et al., 2004). When the chicken genome (GGA) was first published, megabase-sized regions were assigned to the W chromosome, which contain dozens of annotated genes, including *CENT3*, *ARRDC3*, *ELL2* and *FBN2* (Hubbard et al., 2005).

However, this assignment was made on the basis that they contained repeats that were presumed to be W-specific. These repeats have since been shown to be shared with

other chromosomes, including the Z. According to Ensembl (v.37–Feb. 2006) the only regions of GGAW that can be considered W-specific are bases 1–195,831; 4,895,452–4,916,845, and all of the random chromosome W sequences. The task now is to identify where in the chicken genome these putative ‘W chromosome’ sequences are located.

To do this we examined a 2.5-Mb region of the chicken genome that was originally assigned to the W chromosome (GGAW bases 1,289,001–3,878,865). We mapped five BACs from within this region, containing a total of 17 annotated genes (Table 1). The position of BAC probes on the W chromosome according to the chicken genome database is presented in Fig. 1.

Materials and methods

Five chicken genomic DNA BAC clones (Table 1) from the region of interest (Karolchik et al., 2003) were ordered from BACPAC Resources Centre at Children’s Hospital Oakland Research Institute (<http://bacpac.chori.org>). The BAC inserts ranged from ~155 to ~220 kb, and the vector used was pTARBAC2.1. The BAC clones were labeled with digoxigenin-11-dUTP (Roche Applied Science, USA) by nick translation, pre-annealed with 1–3 µg boiled female chicken

Request reprints from Rami Stiglec
Comparative Genomics Group, Research School of Biological Sciences
The Australian National University
Canberra, ACT 0200 (Australia)
telephone: +61 2 6125 2371; fax: +61 2 6125 4891
e-mail: rami.stiglec@anu.edu.au

Fig. 1. Graphic representation of chicken genome database for GGA W chromosome bases 1,289,001–3,878,865. Grey areas are gene-rich regions. Bars depict localization of the five BAC clones (a–e) in this sequence.

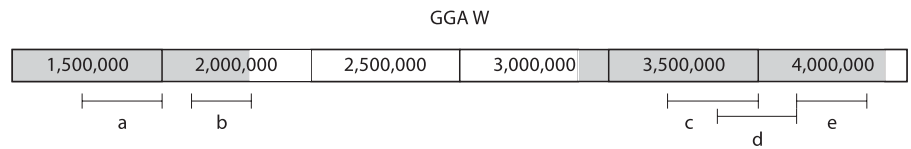


Table 1. The five mapped BAC clones and their gene content

Probe	BAC ID	Gene names	GenBank references
a	CH261-178A20	<i>CENT3</i>	XM_424696
		<i>NP_981951.1</i>	NM_001031608
		<i>POLR3G</i>	XM_424697
		<i>Q7Z3D4</i>	NM_001031414
		<i>MASS1</i>	XM_429120
b	CH261-162L9	<i>MASS1</i>	XM_429120
		<i>ARRDC3</i>	XM_424699
c	CH261-48M12	<i>KIAA0372</i>	XM_424706
		<i>NP_937793.1</i>	NM_001031415
		<i>NP_775498.1</i>	NM_424710
		<i>RHOBTB3</i>	XM_429123
		<i>GLRX</i>	NM_205160
		<i>ELL2</i>	XM_424711
d	CH261-60A13	<i>ELL2</i>	XM_424711
		<i>PCSK1</i>	XM_424712
e	CH261-118K22	<i>CAST</i>	XM_424713
		<i>ARTS-1</i>	XM_424714
		<i>LRAP</i>	AJ851612
		<i>FBN2</i>	XM_424715

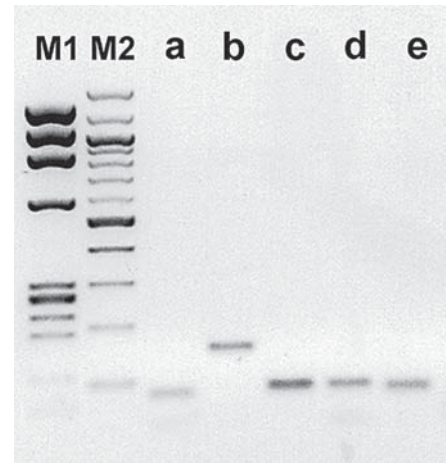


Fig. 2. PCR confirmation of the five BAC clones (a–e). M1: DNA Molecular Weight Marker IX (72–1,353 bp), Roche Applied Science, USA; M2: 100-bp DNA ladder (100–1,517 bp), New England Biolabs.

Table 2. BAC gene-specific primers, annealing temperatures (T_A) and product sizes

Genes	Primers	T_A	Product size
<i>CENT3</i>	F: GAGAGCCTTGGGTTTTGATG R: GGTGATCTTTCCTGTTGCTTC	54°C	85 bp
<i>ARRDC3</i>	F: GCAGCCATTTACCAAACACA R: CAAGGATTGAAGGGGAAACA	54°C	160 bp
<i>ELL2</i>	F: CCAAATCAAACAGCAGGACA R: TTGTGGCAGCCTCTCTTTT	54°C	92 bp
<i>FBN2</i>	F: GTGGTAACGGCAATGGCTAC R: CACCTACACCTGGGGAGAAA	54°C	84 bp

whole-genomic DNA and hybridized to ZW female chicken metaphase chromosomes obtained from chicken embryo fibroblast cultures. Hybridization was detected with Cy3 conjugated to anti-digoxigenin antibodies (Roche Applied Science, USA). Chromosomes were counterstained with DAPI (Sigma laboratories, Australia) and mounted with vectashield (Vector Laboratories). The identity of all probes was confirmed with BAC-specific PCR amplifications, using primers designed from genes within the BACs, generating PCR fragments ranging from 84 to 160 bp (Table 2 and Fig. 2).

Results

The physical mapping data for probes a to e are presented in Fig. 3. All probes were localized between proximal and medial positions on chromosome Z_p, which is identifiable by its presence as a single chromosome on female metaphase chromosome spreads, and also by its size (the fifth largest macrochromosome) and morphology (submetacentric). Specific signals on both chromatids of the Z chromosome were observed in more than 85% of cells hybridized.

Discussion

Five putative 'W chromosome' BACs (probes a to e) localized only to the Z chromosome. No signal was seen on the W chromosome for any BAC probe, indicating that these BACs are all Z-specific rather than W-specific. The genes contained in this region are not, therefore, located on the W chromosome as previously thought, and are unlikely to contribute to female sex determination. However, the position of the 17 genes contained on the five Z-specific BACs on Z_p would be related to a dosage-sensitive function in bird sex determination (McQueen et al., 2001).

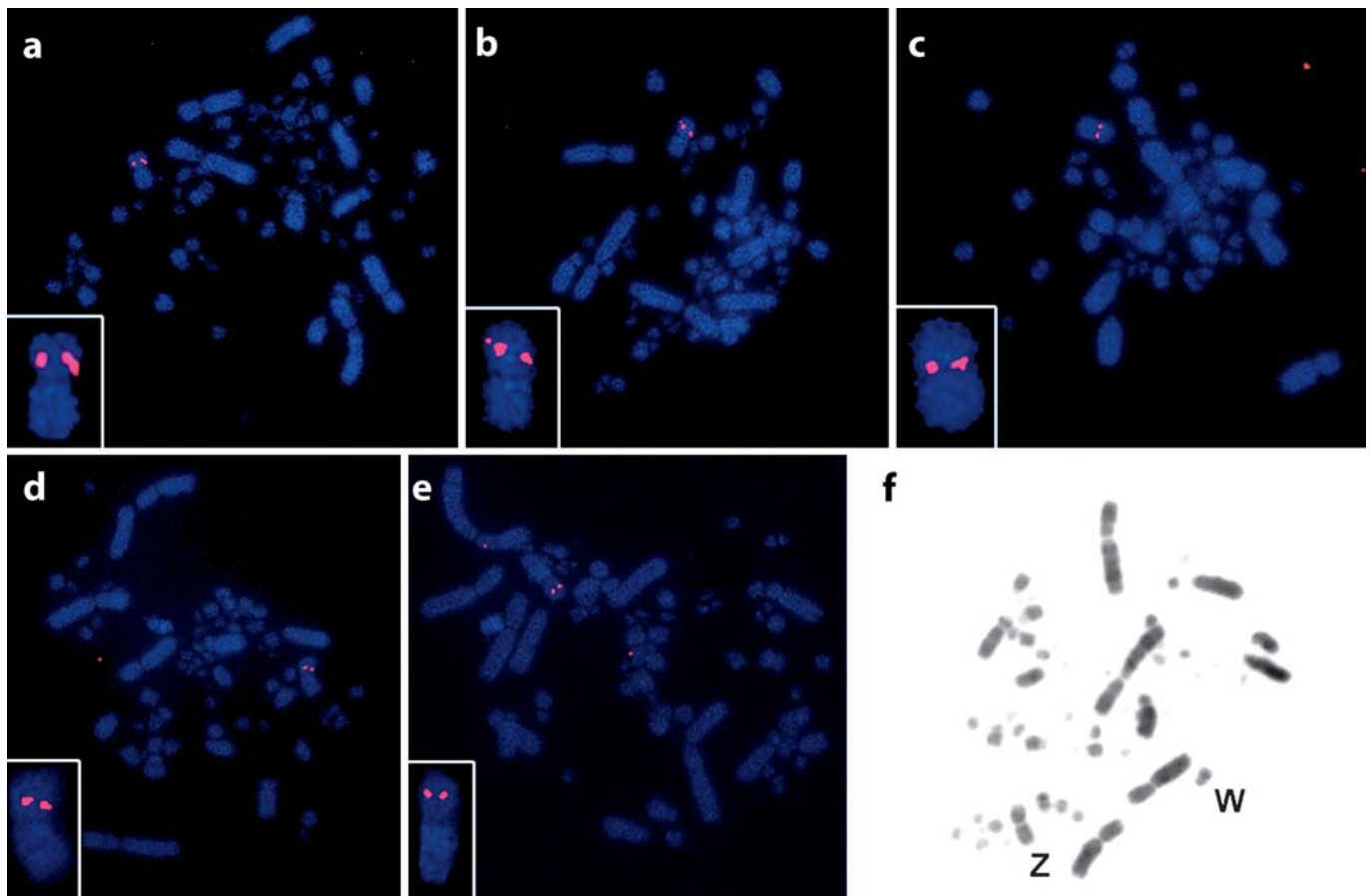


Fig. 3. Female chicken metaphase spreads showing hybridization of the five BAC clones (a–e) to the Z chromosome (present in only one copy in female chicken). Insert: Z chromosome. (f) Inverted female chicken metaphase spread with the Z and W chromosomes labeled.

This work demonstrates that the practice of assigning genomic regions based solely on repetitive sequences has major flaws. As this region of the chicken genome contains at least 17 genes, our re-assignment corrects a number of

gene mis-assignments. These reassignments contribute to the accuracy of the chicken genome database, enhancing its role as an important resource for comparative genome analysis.

References

- Hubbard T, Andrews D, et al: Ensembl 2005. *Nucleic Acids Res* 33:D447–D453 (2005).
- Karolchik D, Baertsch R, et al: The UCSC Genome Browser Database. *Nucleic Acids Res* 31:51–54 (2003).
- McQueen HA, McBride D, et al: Dosage compensation in birds. *Curr Biol* 11:253–257 (2001).
- Reed KJ, Sinclair AH: *FET-1*: a novel W-linked, female specific gene up-regulated in the embryonic chicken ovary. *Mech Dev* 119 (suppl 1): S87–S90 (2002).
- Smith CA, Sinclair AH: Sex determination in the chicken embryo. *J Exp Zool* 290:691–699 (2001).
- Smith CA, Sinclair AH: Sex determination: insights from the chicken. *Bioessays* 26:120–132 (2004).
- Yamada D, Koyama Y, et al: Comprehensive search for chicken W chromosome-linked genes expressed in early female embryos from the female-minus-male subtracted cDNA macroarray. *Chromosome Res* 12:741–754 (2004).