UC Davis
UC Davis Previously Published Works

Title

Genetic variation exists for telomeric array organization within and among the genomes of normal, immortalized, and transformed chicken systems

Permalink

https://escholarship.org/uc/item/7n8712bg

Journal

Chromosome Research: Cytogenetics, genomics, chromatin and the nucleus, 17(8)

ISSN

1573-6849

Authors

O'Hare, Thomas H.
Delany, Mary E.

Publication Date

2009-12-01

DOI

10.1007/s10577-009-9082-6

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Chromosome Research (2009) 17:947–964
DOI 10.1007/s10577-009-9082-6

Genetic variation exists for telomeric array organization within and among the genomes of normal, immortalized, and transformed chicken systems

Thomas H. O'Hare · Mary E. Delany

Received: 16 July 2009 / Revised: 3 September 2009 / Accepted: 28 September 2009 / Published online: 5 November 2009
© The Author(s) 2009. This article is published with open access at Springerlink.com

Abstract This study investigated telomeric array organization of diverse chicken genotypes utilizing in vivo and in vitro cells having phenotypes with different proliferation potencies. Our experimental objective was to characterize the extent and nature of array variation present to explore the hypothesis that mega-telomeres are a universal and fixed feature of chicken genotypes. Four different genotypes were studied including normal (UCD 001, USDA-ADOL Line 0), immortalized (DF-1), and transformed (DT40) cells. Both cytogenetic and molecular approaches were utilized to develop an integrated view of telomeric array organization. It was determined that significant variation exists within and among chicken genotypes for chromosome-specific telomeric array organization and total genomic-telomeric sequence content. Although there was variation for mega-telomere number and distribution, two mega-telomere loci were in common among chicken genetic lines (GGA 9 and GGA W). The DF-1 cell line was discovered to maintain a complex derivative karyotype involving chromosome fusions in the homozygous and heterozygous condition. Also, the DF-1 cell line was found to contain the greatest amount of telomeric sequence per genome (17%) as compared to UCD 001 (5%) and DT40 (1.2%). The chicken is an excellent model for studying unique and universal features of vertebrate telomere biology, and characterization of the telomere length variation among genotypes will be useful in the exploration of mechanisms controlling telomere length maintenance in different cell types having unique phenotypes.

Keywords telomere · chicken · FISH · DT40 · DF-1 · Red Jungle Fowl

Abbreviations

ALV	Avian leukosis virus
BAC	Bacterial artificial chromosome
CE	Chicken embryo
CEF	Chicken embryo fibroblast
DAPI	4′,6-diamidino-2-phenylindole
E	Embryonic day or days of embryogenesis
ETS	External transcribed spacer
FISH	Fluorescence in situ hybridization
FPC	Finger-printed contigs
GGA	*Gallus gallus*
MHC	Major histocompatibility complex
PBS	Phosphate buffered saline
PFGE	Pulsed-field gel electrophoresis
PNA	Peptide nucleic acid

Responsible Editor: Irina Solovei.

T. H. O'Hare (✉) · M. E. Delany
Department of Animal Science,
University of California, Davis,
One Shields Avenue,
Davis, CA 95616, USA
e-mail: thohare@ucdavis.edu

M. E. Delany
e-mail: medelany@ucdavis.edu

rDNA	Ribosomal DNA
SSC	Sodium salt citrate
4T	4x SSC, 0.05% Tween-20
TNB	100 mM TrisHCl, 150 mM NaCl, 0.5% blocking reagent (Roche)
TNT	100 mM TrisHCl, 150 mM NaCl, 0.05% Tween-20
UCD	University of California, Davis
USDA-ADOL	United States Department of Agriculture, Avian Disease and Oncology Laboratory

Introduction

Telomeres were initially defined as the ends of linear chromosomes having morphologically and functionally distinct features (Muller 1938; McClintock 1941). The telomere is now known to consist of a conserved tandemly repeated sequence $(TTAGGG)_n$ bound with specific proteins. The conserved sequence can also be found at interstitial locations perhaps marking sites of chromosome fusions (Wells et al. 1990; Lee et al. 1993; Nanda and Schmid 1994; Dahse et al. 1997; Nanda et al. 2002; Hartmann and Scherthan 2004; Bolzán and Bianchi 2006). Although vertebrate telomeres vary in the size of the array, in general, their range is 10–20 Kb (Davis and Kipling 2005). However, some vertebrates show array size extremes, e.g., mouse and chicken. Interestingly, the laboratory inbred mouse strains (*Mus musculus*) exhibit telomeric arrays of 30–150 Kb, while the wild mouse species (*Mus spretus*) has shorter telomeres of 5–15 Kb (Kipling and Cooke 1990; Prowse and Greider 1995; Coviello-McLaughlin and Prowse 1997; Hemann and Greider 2000; Kim et al. 2003). In the chicken, there are three classes of telomeric arrays (Class I, interstitial; Class II and III, terminal), and the terminal telomeres range from approximately 20 Kb to several Mb (Delany et al. 2000; Nanda et al. 2002; Rodrigue et al. 2005; Delany et al. 2007). The largest telomeric arrays (Class III) were termed "ultra-long" telomeres as analyzed by molecular approaches (Delany et al. 2000) and "mega-telomeres" via cytogenetic approaches (Delany et al. 2007), but all evidence suggests their equivalence and therefore herein are referred to as mega-telomeres. The variation for this distinctive class of telomeric arrays among different chicken genotypes has not been previously established. Further, the control mechanisms regulating and maintaining different array sizes among chromosomes within genomes is not well understood.

The inheritance of mega-telomere arrays was studied in a highly inbred chicken line (University of California, Davis (UCD) 003) wherein the arrays exhibited a hyper-variable inheritance pattern suggestive of a high degree of recombination (Rodrigue et al. 2005). In addition, these arrays were mapped to four autosomes and one sex chromosome (one mega-telomere array per chromosome). The female-specific array was sized as 2.8 Mb and mapped to the q arm of GGA W, the female-specific sex chromosome (Rodrigue et al. 2005; Delany et al. 2007). The autosomal mega-telomere arrays mapped to chromosomes 9, 16, and 28, with the fourth locus unknown. The in-common features of the chromosomes with mega-telomeres at least in the case of GGA W, 9, and 16 was the presence of other repetitive sequence elements, in addition to belonging to the size class of intermediate chromosomes and microchromosomes, rather than the macrochromosomes (Delany et al. 2007).

Telomere length is a critical genetic mechanism governing cell survival. Short telomeres are recognized during the cell cycle as damaged DNA which triggers cell cycle arrest or apoptosis. Telomeres also provide for maintenance of chromosomal genetic material (Hemann et al. 2001; Shawi and Autexier 2008). In both chicken and human, telomerase maintains the telomeres and exhibits a variable activity profile, being absent in differentiated somatic cells and active in embryonic and transformed cells (Taylor and Delany 2000; Swanberg and Delany 2003). Although the chicken is a well-studied organism for telomere biology (Delany et al. 2003; Swanberg and Delany 2006), currently, except for telomerase, there is no knowledge regarding additional mechanisms for telomere maintenance in chicken cells. Telomerase-negative cells including immortalized and transformed cells in other organisms, e.g., human and mouse, have been shown to adopt other methods of lengthening telomeres in the absence of telomerase, known as ALT or alternative lengthening of telomeres (Reddel et al. 2001; Henson et al. 2002; Scheel and Poremba 2002; Blasco 2008; Royle et al. 2008).

Beyond the study of one inbred chicken line, there has been no analysis to establish the uniformity of telomeric array profiles within and among different

genotypes. Improved understanding of telomere length profiles in diverse chicken genotypes will contribute toward our understanding of the telomere and telomerase pathway of chicken cells, in normal as well as immortalized and transformed cellular phenotypes, with insight into dysregulation of the pathway in the abnormal cell types. With this in mind, the main objective of this study was to establish the extent and degree of variation for telomeric array length at several levels: intra-genomic, inter-individual, and inter-genotype. Diverse chicken genotypes encompassing normal, immortalized, and transformed phenotypes were utilized, specifically to examine the status of mega-telomeres among genetic lines and established cell lines. The hypothesis for this study was that mega-telomere array loci are universal and thus, it was predicted that all genetic stocks would be identical for mega-telomere number and distribution. The telomeric array profiles among the diverse chicken genotypes were assessed using both cytogenetic and molecular methods to allow for an integrated view of telomeric array variation from the individual chromosomal to the total genomic level. Two genetic lines were studied, UCD 001 (Red Jungle Fowl, *Gallus gallus gallus*), the sequenced chicken genome genetic line (International Chicken Genome Sequencing Consortium (ICGSC) 2004) and the species that is the ancestor to domestic chicken breeds (Fumihito et al. 1994), and United States Department of Agriculture, Avian Disease and Oncology Laboratory (USDA-ADOL) Line 0 (Single Comb White Leghorn, *Gallus gallus domesticus*), a genetic line developed to be free of endogenous retroviruses (Bacon et al. 2000). In addition, two well-utilized chicken cell lines, DF-1 (immortalized chicken embryo fibroblast (CEF)) and DT40 (transformed B-cell lymphoma), were also studied.

Materials and methods

Genotypes and chromosome procedures

Chromosomes were harvested according to Rodionov et al. (2002) from eight chicken embryos (males and females) at E4.5, of the inbred UCD 001 Red Jungle Fowl line ($F \approx 0.90$; Delany and Pisenti 1998) and from the Single Comb White Leghorn USDA-ADOL Line 0 (Bacon et al. 2000), hereafter referred to as ADOL Line 0. In addition, chromosomes were harvested according to Delany et al. (2007) from three CEF cultures (Swanberg and Delany 2003) at early to mid-passage (P2 to P13) from two single UCD 001 male embryos and a single female embryo from the inbred UCD 003 Single Comb White Leghorn line ($F > 0.99$; Abplanalp 1992). Chromosomes were also harvested (Chang and Delany 2004) from two established cell lines including DT40, a transformed B-cell line derived from a bursal lymphoma of an avian leukosis virus (ALV)-infected SC Hy-line female chicken (Baba et al. 1985) and DF-1 (Himly et al. 1998, ATCC CRL-12203), an immortalized CEF line derived from ADOL Line 0 embryos. Chromosome slides were prepared and stored according to Delany et al. (2007).

Chromosome-specific and telomere-sequence probes

Most of the probes (Table 1) used to identify specific chromosomes were from large insert bacterial artificial chromosome (BAC) clones (Lee et al. 2003; Delany et al. 2007). One of the GGA 9 probes was a chicken 5S rRNA plasmid clone (Daniels and Delany 2003), and the probe for GGA 16 was the 5′ external transcribed spacer (ETS) region of the 18S-5.8S-28S rRNA gene (Delany and Krupkin 1999). The probes were labeled using Nick Translation (Abbott Molecular or Invitrogen) with a fluorochrome-dUTP, i.e., Spectrum Red (Abbott Molecular), Spectrum Orange (Abbott Molecular), Texas Red (Invitrogen), or Cy3 (GE Healthcare). Alternatively, probes were labeled with digoxigenin using the DIG-Nick Translation kit (Roche Applied Science) and detected by a secondary anti-digoxigenin antibody (Roche Applied Science) conjugated with either Rhodamine or Fluorescein. A telomere-peptide nucleic acid (PNA) fluorescein probe (Applied Biosystems) was used to identify telomeric sequence repeats.

Fluorescence in situ hybridization (FISH)

Slides were removed from −80°C at least 6 h before use to allow for equilibration to room temperature. For telomeric sequence-only hybridizations, 24 µl of telomere-PNA probe was applied to the slide which was covered with a Hybrislip (Research Products International), placed in 65°C slide moat for 5 min, and then immediately placed in a humid chamber at room temperature for 30 min. Post-hybridization

Table 1 Details of chicken chromosome-specific probes[a]

GGA	Clone identification	Features[b]	Insert Size (Kb)[c]	Location/Size (Mb)[d]	References
6	CH261-169D14	SCD (AJ297918/X60465)	226[I]	18.5/37.4	Pitel et al. 1998
7	CH261-95H15	SP5 (NM_001044684)	158[I]	19.7/38.4	
8	CH261-84K8	ZNF326 (NM_001006533)	235[I]	15.7/30.7	
9	TAM31-29A21	TR (AY312571)	ND	21.5/25.6	Delany and Daniels 2003
	CH261-25N18	ATP13A4 (NM_001031314)	187[I]	14.1/25.6	
	CH261-33G6	SLC25A36 (NM_001007960)	184[I]	7.7/25.6	
	5S rDNA	(AF419700)	2.1	1.9/25.6	Daniels and Delany 2003
10	TAM33-42N22	NEO1 (U07644)	179[II]	1.3/22.6	
11	TAM32-22B17	ADL210 (G01630)	181[II]	12.8/21.9	
12	TAM32-43M12	MCW198 (G31980)	118[II]	12.7/20.5	
16	TAM31-44G24	MHC-B	125	-	Shiina et al. 2007
	TAM31-66A9	MHC-Y	115[III]	-	
	ETS rDNA	NOR	3	-	Delany and Krupkin 1999
26	CH261-126M22	ARL8A (NM_001012868)	201[I]	0.2/5.1	
28	TAM32-4G3	ADL299 (G01751)	167[II]	4.3/4.5	
W	TAM32-55E18	CW01 (D85614)	ND	-	Ogawa et al. 1997, Delany et al. 2007

[a] BAC locations and features were obtained from US Poultry Genome Project 'Database of BACs Assigned to Chicken Genes and Markers' (http://poultry.mph.msu.edu/resources/resources.htm, May 2006 version) and/or UCSC Genome Browser (http://genome.ucsc.edu, Chicken May 2006 assembly)

CH Children's Hospital Oakland Research Institute, *CH261* EcoRI BAC library

TAM Texas A&M University, *TAM31* BamHI, *TAM32* EcoRI, *TAM33* HindIII BAC libraries (Lee et al. 2003, Ren et al. 2003)

ETS external transcribed spacer of the 18S-5.8S-28S rRNA gene repeat (rDNA)

[b] Features indicate genes/markers and GenBank accession numbers (in parentheses)

TR telomerase RNA, *MHC* major histocompatability complex, *NOR* nucleous organizer region, *SCD* stearoyl-CoA desaturase, *SP5* Sp5 transcription factor, *ZNF326* zinc finger protein 326, *ATP13A4* ATPase type 13A4, *SLC25A36* solute carrier family 25, member 36, *NEO1* neogenin, *ARL8A* ADP-ribosylation factor-like 8A, *CW01* non-repetitive chromosome W DNA marker

ADL210, ADL299, and MCW198 are sequence tagged sites

[c] Clone insert sizes were determined in previous research (references as indicated) or by one of the following three ways: [I] Insert sizes were obtained from the UCSC Genome Browser (http://genome.ucsc.edu); [II] Insert sizes were estimated using the UCSC Genome Browser and Chicken FPC (http://www.bioinformatics.nl/gbrowse/cgi-bin/gbrowse/ChickFPC) as follows: BAC inserts of known size (Kb) in the UCSC Genome Browser were used to estimate the size of BAC inserts lacking size information. A ratio of Kb/u was calculated from the BAC inserts of known size, the units (u) value was obtained from the chicken FPC database. This ratio was calculated from the average of three BACs in the same region and overlapping the BAC of interest within chicken FPC database. The FPC value of the BAC of interest was then multiplied by the ratio to obtain Kb size; [III] Insert size provided by Dr. Marcia Miller (City of Hope Medical Center, Duarte CA, personal communication); *ND* not determined, insert size could not be determined because the BAC was not listed in the databases

[d] Location refers to the start position (in Mb) of the BAC or gene/marker on the chromosome in the May 2006 chicken assembly (UCSC Genome Browser). Size refers to the total assembled sequence for the chromosome. The dash (-) indicates that incomplete assembly of the chromosome does not allow for Mb location and chromosome size estimates

washes included the following: 15 min in 1x phosphate buffered saline (PBS)/0.1% Tween-20 at 57°C, 1 min in 2x sodium salt citrate (SSC)/0.1% Tween-20 at room temperature, and rinse in 1x PBS. Thirty microliters of Vectashield Mounting Medium with 4′,6-diamidino-2-phenylindole (DAPI; Vector Laboratories) diluted 2:15 with Vectashield Mounting Medium (Vector Laboratories) were placed on the slide and covered with a glass coverslip. The slides were stored flat at 4°C until image capture which took

place within 24 h. For BAC-probe hybridizations, the slides were heat treated at 65°C in a dry incubator for 12 to 24 h and dehydrated in 70%, 80%, and 95% ethanol for 5 min each. The preparations were denatured using 70% deionized formamide at 66°C for 1 min 10 s and immediately placing the slides in ice cold 70% ethanol for 5 min followed by 70%, 95%, and 100% ethanol rinses (on ice) for 5 min each. Probes were added to the slide in a mixture containing 5 µl BAC-probe, 15 µl hybridization mix (50% deionized formamide, 0.2x SSC, 7.5 µg sheared chicken DNA, 6.7% dextran sulfate), 20 µl telomere-PNA probe (or 15 µl water), covered with a Hybrislip, and placed in 37°C slide moat overnight. Post-hybridization washes included 1x PBS/0.1% Tween-20 at 57°C for 15 min, 2x SSC/0.1% Tween-20 at room temperature for 1 min, and 1x PBS rinse. When using anti-digoxigenin-rhodamine (or -fluorescein), the following procedures were included: 40 µl TNB (100 mM TrisHCl, 150 mM NaCl, 0.5% blocking reagent (Roche)) were added to the slide which was covered with a Hybrislip and placed in 37°C slide moat for 30 min followed by incubation with 35 µl anti-digoxigenin (3.5 µl anti-digoxigenin and 32.5 µl TNB) covered by a Hybrislip at 37°C for 30 min, followed by washes in 4T, TNT (100 mM TrisHCl, 150 mM NaCl, 0.05% Tween-20), and TNT 5 min each, with a final rinse in 1x PBS. The slides were DAPI stained and stored as described above.

Cytogenetic analysis

Images were captured using SimplePCI 6.0 (Hamamatsu Corporation) with an Olympus BX60 microscope or Applied Imaging CytoVision Genus 3.93 (Genetix) with an Olympus BX41 microscope. As in prior work (Delany et al. 2007), the green telomeric sequence fluorescence in situ hybridization (FISH) signals were standardized using the image capture software, i.e., telomeric sequence fluorescein signals were adjusted (reduced) to the point wherein GGA 1 and GGA 2 interstitial telomeric sequences were not visible. This adjustment left visible only the mega-telomere signals, which were counted in each cell. A minimum of 20 cells were analyzed for each sample to determine mega-telomere counts per individual and to calculate descriptive statistics (mode, mean, standard deviation, and range). When determining chromosome location for mega-telomeres, 20–40 cells were analyzed.

Telomeric array sizing

Horizontal gel electrophoresis-class I and II arrays

Blood (0.3 ml in 0.2 ml anticoagulant 0.07 M NaCitrate/0.07 M NaCl solution) was collected from a female UCD 001 chicken. The DNA was extracted from the blood sample and from DT40 and DF-1 cells using the QIAamp DNA Blood Mini Kit (Qiagen). DNA concentrations were determined using a Nano-Drop 1000 Spectrophotometer (Thermo Scientific). The DNA was digested using HinfI and RsaI. DNA fragments (150 ng per lane) were separated using standard electrophoresis conditions (0.7% agarose, 1x TAE, 55 V, 4 h) to resolve Class I (0.5–10 Kb, interstitial) and Class II (10–40 Kb, terminal) telomeric arrays (Delany et al. 2000; Rodrigue et al. 2005). After electrophoresis, the agarose gel was imaged using a Florimager 595 (GE Healthcare). The DNA was then transferred to a positively charged nylon membrane (Roche) by Southern blotting using 20x SSC; the resulting membrane was utilized for telomere probe hybridization (following the Roche TeloTAGGG Telomere Length Assay protocol). The chemiluminescence signal from the membrane was exposed on Lumi-Film Chemiluminescent Detection Film (Roche). The telomeric arrays were sized by comparing to the DIG-Molecular weight marker included in the Roche telomere length kit.

Pulsed-field gel electrophoresis (PFGE)-class III arrays

Blood was collected from six (three males and three females) UCD 001 chickens and four (two males and two females) UCD 003 chickens. Erythrocyte counts were determined for each sample using a hemacytometer. Cells from two UCD 001 CEF males, DT40, and DF-1 were also counted using a hemacytometer. Agarose plugs were created using Bio-Rad disposable plug molds according to manufacturer recommendations (Bio-Rad CHEF-DR II manual) with 3 µg DNA (based on cell counts and assuming 2.5 pg DNA/cell) per plug and incubated at 55°C in 5 ml wash buffer (10 mM TrisHCl pH 7.6, 25 mM EDTA pH 8.0, 1% sarkosyl) with 150 µl 20 mg/ml proteinase K (New England Biolabs) overnight. The plugs were then washed three times with suspension buffer (10 mM TrisHCl pH 7.6, 20 mM EDTA pH 8.0) at room

temperature. All plugs were sliced in thirds (1 μg); slices were individually digested with 4 μl Hae III (New England Biolabs) in 356 μl 1x NEBuffer 2 (New England Biolabs) for 12–16 h at 37°C then placed directly in the gel well. Three pulsed-field gel electrophoresis (PFGE) conditions were utilized to resolve subcategories of Class III (50 Kb to Mb arrays, Delany et al. 2000) telomeric array lengths: conditions 1 (50–800 Kb) and 3 (1–3 Mb) as described by Rodrigue et al. (2005), and condition 4 (this study, 3.5–5.7 Mb) to determine the size of a female-specific telomeric array. The details of condition 4 include 2 V/cm, 1,200–1,800 switch time, 72 h, 0.8% Megabase agarose (Bio-Rad), and 1x TAE (40 mM Tris, 20 mM acetic acid, 1 mM EDTA), with inclusion of an *Schizosaccharomyces pombe* marker (Bio-Rad). After electrophoresis, Southern blot and hybridization was performed as described above. The agarose gel was imaged before Southern blot using a Fujifilm FLA-5100 imaging system to capture an image of the molecular makers to size the telomeric arrays on the resulting film.

Total telomeric sequence content by slot blot analysis

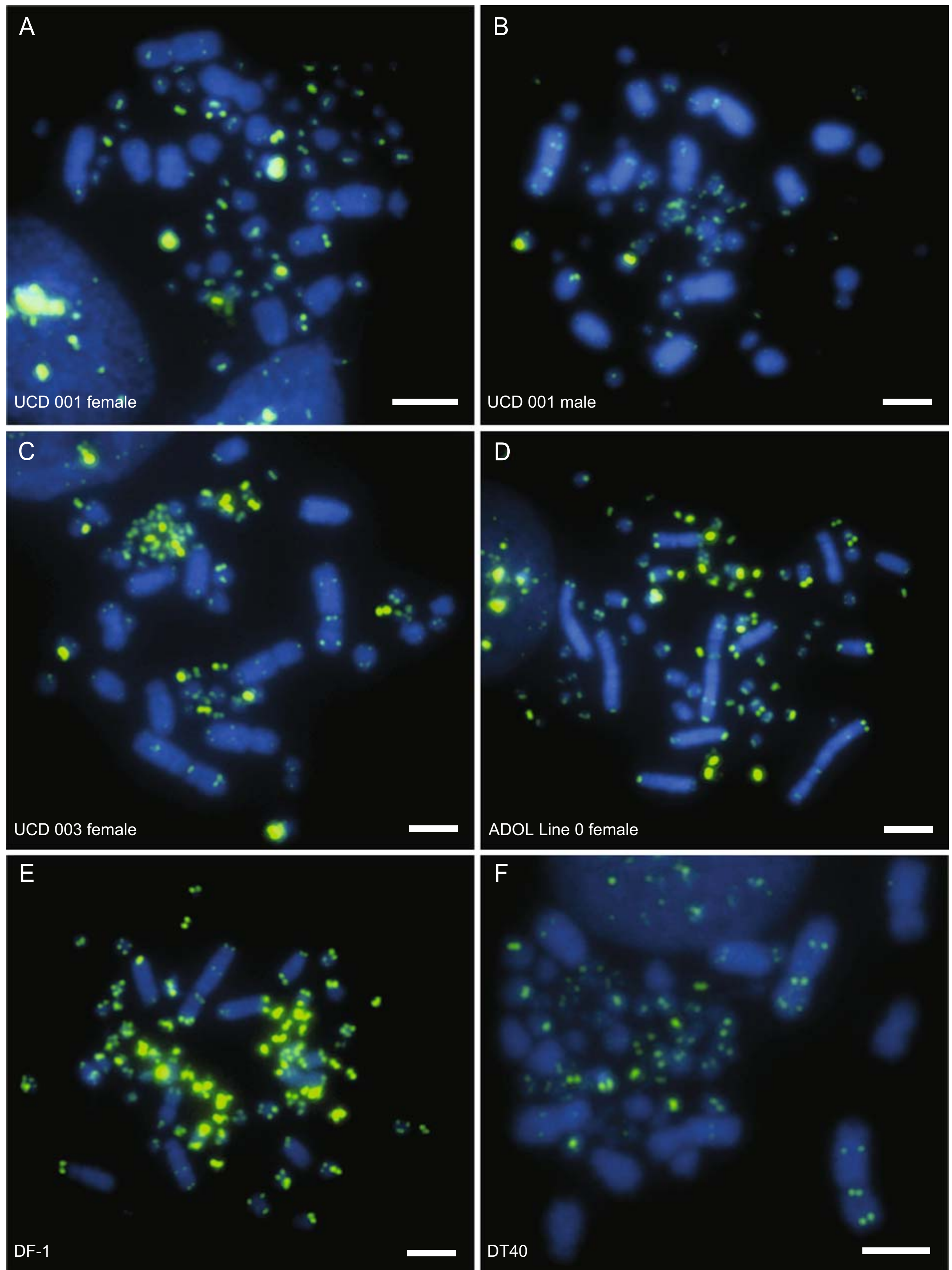
One hundred nanograms of DNA from one UCD 001 female and the DF-1 and DT40 cell lines (in triplicate) were applied to a nylon membrane (Roche) using a Schleicher & Schuell Minifold II slot blot apparatus following the Bio-Rad DNA Dot/Slot Blotting protocol (from Zeta-Probe GT Blotting Membranes Instruction Manual) and Delany et al. (2000) procedures. A telomeric sequence standard curve was created using a G-rich strand oligonucleotide (5′-TTAGGG-3′)$_7$. Following application of the samples and TTAGGG-standards, the membrane was rinsed in 2x SSC, baked at 80°C for 1 h, and hybridized according to the Roche TeloTAGGG telomere length assay procedures. The chemiluminescence signal produced on the membrane was captured using a Fujifilm FLA-5100 imaging system. Signal intensities were calculated using Fujifilm MultiGauge software (version 3.0). The software calculates an intensity value for the slot blot bands, and the sample values were compared to the known concentration standards to determine the telomeric sequence concentration of the UCD 001, DT40, and DF-1 samples. The percentage of total telomeric sequence per genotype was calculated by dividing the telomeric sequence concentration of the sample by 100 ng (DNA concentration loaded in each sample well).

Results

Genotype-specific variation for telomeric array profiles was investigated by both cytogenetic and molecular evaluation methods. Overall TTAGGG-hybridization intensity patterns (terminal and interstitial sites), the number of mega-telomere arrays, and the map location for the mega-telomere arrays were studied and integrated with molecular sizing data. Mapping was conducted by multi-color FISH using chromosome-specific probes (Table 1). Mega-telomeres were previously mapped in UCD 003, and those map locations were used as a starting point for this analysis (Rodrigue et al. 2005; Delany et al. 2007).

Figure 1 shows representative results illustrating that significant variation exists for telomeric array organization among the genotypes. The cytogenetic results indicated that the Single Comb White Leghorn lines UCD 003 and ADOL Line 0 had relatively more telomeric sequence than UCD 001 (Fig. 1C, D compared to Fig. 1A, B); all three genetic lines showed less overall telomeric sequence by FISH analysis than DF-1 but more than DT40 (Fig. 1E, F, respectively). Molecular analysis was employed to expand on the

Fig. 1 Comparative telomeric array organization within and among diverse chicken genotypes illustrates intra-genomic, inter-individual, and inter-genotype variation. The genotypes shown include the Red Jungle Fowl line UCD 001 (**A**, **B**), Single Comb White Leghorn lines UCD 003 (**C**), and ADOL Line 0 (**D**), as well as two cell lines DF-1 (**E**) and DT40 (**F**). Cells shown in **A**, **C**, and **D** are from females and **B** is from a male. The DF-1 line was created from a group of ADOL Line 0 embryos presumably including both males and females, and the DT40 line was created from a bursal lymphoma from a female bird (Hyline SC). The chromosomes were hybridized with telomere-PNA probe (*green*) and counterstained with DAPI (*blue*). This figure provides an overall view of the telomeric array profile for each genotype in terms of interstitial and terminal telomeric arrays including the larger class of arrays, the mega-telomeres. The images were adjusted to a similar degree by adjusting GGA 1 interstitial signals and avoiding oversaturation of mega-telomere arrays. The cytogenetic analysis provided a qualitative view of telomeric array distribution and organization variation within cells, between individuals, and among genotypes. It was apparent that there were genotype-specific differences not only for distribution but also for amount of TTAGGG-sequence with the general pattern of DT40<UCD 001<ADOL Line 0≈UCD 003<DF-1. *Scale bar*, 5 μm ▶


A
UCD 001 female
B
UCD 001 male
C
UCD 003 female
D
ADOL Line 0 female
E
DF-1
F
DT40

qualitative cytogenetic observations wherein array lengths and estimation of total telomeric sequence content were examined.

Telomeric cytogenetics of in vivo genotypes: UCD 001 and ADOL Line 0

Inter-individual variation for the mega-telomere array profile was observed in UCD 001 (Table 2, Fig. 2). In all individuals, one mega-telomere locus (two signals) mapped to GGA 9p, and in all females, a mega-telomere mapped to GGA W (Fig. 2A). Three of four females exhibited three signals, and one female, chicken embryo (CE)-6, exhibited four signals. One female (CE-6) exhibited mega-telomeres mapping to both arms (p and q) of one GGA 9 homolog (Fig. 2B). Three of four males exhibited two mega-telomere array signals, while one male, CE-2, exhibited three signals. The variant male (CE-2) indicated a mega-telomere on one GGA Z homolog (Fig. 2C). The interstitial telomeric array signal profile was similar to what has been observed in UCD 003 (Delany et al. 2007).

The analysis of ADOL Line 0 included one male and one female. The male exhibited eight mega-telomere array signals, and the female exhibited seven signals (Table 2). Mega-telomere arrays were mapped to GGA 9p and GGA 16p in both individuals accounting for four of the signals. The fifth signal in the female was mapped to GGA W (Fig. 3A). The male exhibited a mega-telomere on GGA 2p (Fig. 3B). Chromosome 28 was also tested because this chromosome was positive for a mega-telomere in UCD 003; however, GGA 28 was negative for a mega-telomere in ADOL Line 0 (Fig. 3A). Thus, there remain two unmapped mega-telomere signals (potentially one locus) in the ADOL Line 0 individuals. The interstitial telomeric array signals observed in this genetic line were similar to those observed in UCD 001 and UCD 003 for size (hybridization intensity) and distribution (GGA 1, 2, W).

Telomeric cytogenetics of in vitro genotypes: DT40 and DF-1

The transformed cell line DT40 did not exhibit mega-telomeres and in fact indicated an overall reduced terminal telomere signal profile (Fig. 1F). Interestingly, some of the terminal telomere signals were not evident even when signal intensity was increased through image analysis procedures. The interstitial telomeric array profile pattern appeared similar to that seen in the

Table 2 Descriptive statistics and chromosomal locations of mega-telomeres in two genotypes: UCD 001 and ADOL Line 0 illustrate individual and genotype variation

Genetic line	Individual	Sex	Mega-telomere signals[a]				Mega-telomere chromosome location[b]			
			Mode	Mean	SD	Lo–hi range	9	16	W	Unique locations
UCD 001	CEF-1	Male	2	2.6	0.8	2–4	+	−	NA	
	CEF-2	Male	2	2.4	0.6	2–4	+	−	NA	
	CE-1	Male	2	2.6	0.8	2–4	+	−	NA	
	CE-2	Male	3	3.5	1.2	2–6	+	−	NA	One GGA Z
	CE-3	Female	3	3.5	0.9	2–6	+	−	+	
	CE-4	Female	3	3.3	0.6	3–5	+	−	+	
	CE-5	Female	3	3.2	0.7	2–4	+	−	+	
	CE-6	Female	4	3.6	0.6	3–5	+	−	+	Both arms of one GGA 9
ADOL Line 0	CE-7	Female	7	7.7	1.8	5–12	+	+	+	
	CE-8	Male	8	7.3	1.5	5–10	+	+	NA	GGA 2

CEF chicken embryo fibroblasts, *CE* chicken embryo, − absence of mega-telomere, + presence of mega-telomere, *NA* not applicable

[a] Mega-telomere signals were counted in cells hybridized with the telomere-PNA probe, and mode, mean, range, and standard deviation (SD) were calculated. A minimum of 20 cells were analyzed for each individual

[b] Mega-telomere locations were tested in UCD 001 and ADOL Line 0 for GGA 9, 16, and 28 by chromosome-specific probe hybridization, whereas the macrochromosomes and GGA W were identified by size and DAPI-staining pattern. A chromosome positive for a mega-telomere reflects that one chromosome arm was involved except where indicated (e.g., CE-6)

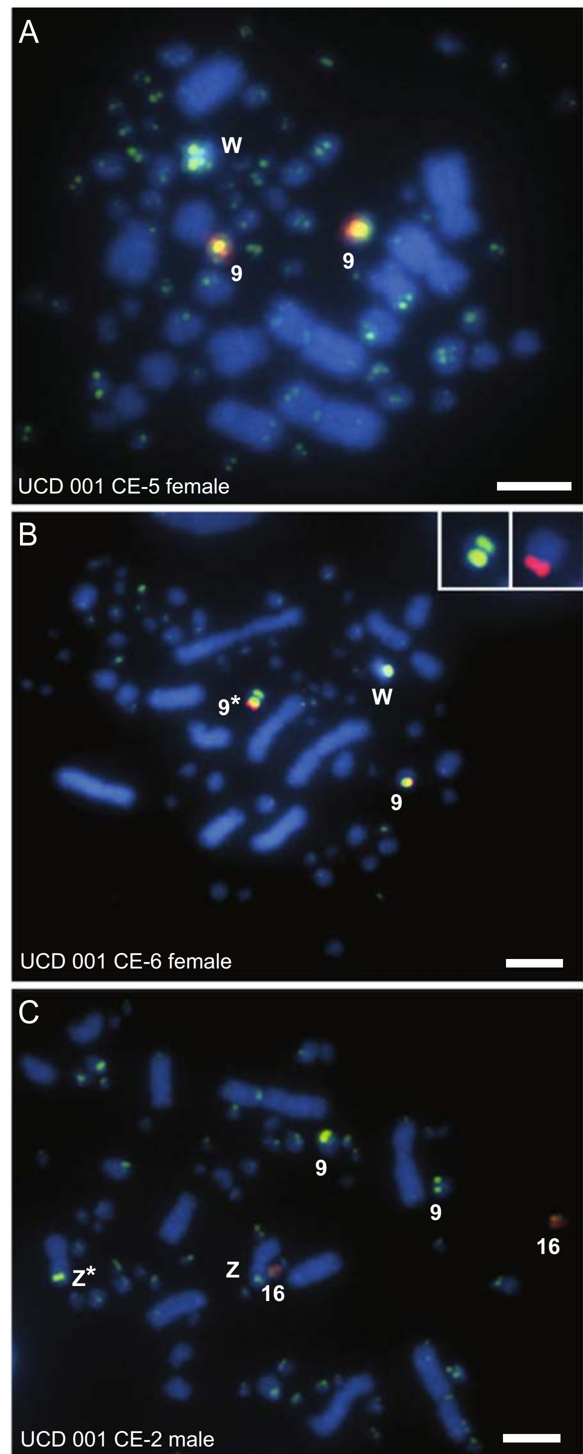


Fig. 2 Chromosomal locations of the mega-telomeres in UCD 001 individuals: in-common and variant loci. **A** UCD 001 female (ZW) cell showing that both GGA 9 homologs (*red* signal, 5S rDNA, see Table 1) have a p arm mega-telomere and that the GGA W (identified by size and DAPI-banding pattern) also possesses a mega-telomere. **B** UCD 001 female cell indicating a mega-telomere on both arms (p and q) of one GGA 9 (*) as well as the p arm of the other homolog, the *inset* illustrates the double mega-telomere GGA 9 with 5S rDNA probe (*red* signal) hybridization at the p arm and mega-telomeres (*green*) at both the p and q arms. **C** UCD 001 male cell showing a mega-telomere on one GGA Z (*) and both GGA 9 homologs. Also indicated in (**C**) is that GGA 16 (*red* signal, external transcribed spacer rDNA, see Table 1) does not have a mega-telomere in this genotype. All images were adjusted to show brightest telomeres only (see "Materials and methods"). *Scale bar*, 5 µm

other genotypes. Although the GGA W exhibited one of the strongest telomere fluorescence signals in the DT40 genome, it was not classified as a mega-telomere, relative to that seen in other genotypes and based on the standard procedure for determining the mega-telomere arrays (see "Materials and methods").

The immortalized cell line DF-1 exhibited a very intense telomeric array fluorescence signal profile overall, with numerous strikingly bright telomere signals (Fig. 1E). During mapping analysis, it was evident that DF-1 has a complex derivative karyotype, and this is described further below. One of the seven chromosomes analyzed was determined to have a mega-telomere, i.e., GGA 16 (Fig. 4A), while the other chromosomes studied (GGA 7, 8, 9, 10, 28, and W) were negative.

Cytogenetics of a derivative karyotype: DF-1

The DF-1 cell line was found to maintain cells of three ploidy levels: haploid (14%), diploid (78%), and tetraploid (8%). Percentages were determined from counts of 553 cells over three cytological preparations. Regardless of ploidy, the cells showed an abnormal, highly derivative karyotype. Seven chromosomes were studied using chromosome-specific probes (Table 1), and five (GGA 10, 16, 28, and W) were found to be involved in chromosome fusions (Fig. 4). In diploid cells, both GGA 16 and 28 were present in the homozygous condition as a part of a derivative chromosome (Fig. 4B), while a GGA 10 fusion chromosome was present in the heterozygous condition (Fig. 4C). GGA W was present in two copies, one derivative and one normal-sized chromosome, and did not exhibit a mega-telomere (Fig. 4A). In haploid cells, either the normal or the derivative copy of GGA W was present, but not both (Fig. 5). Also, Fig. 5 shows that GGA 16 is present in a

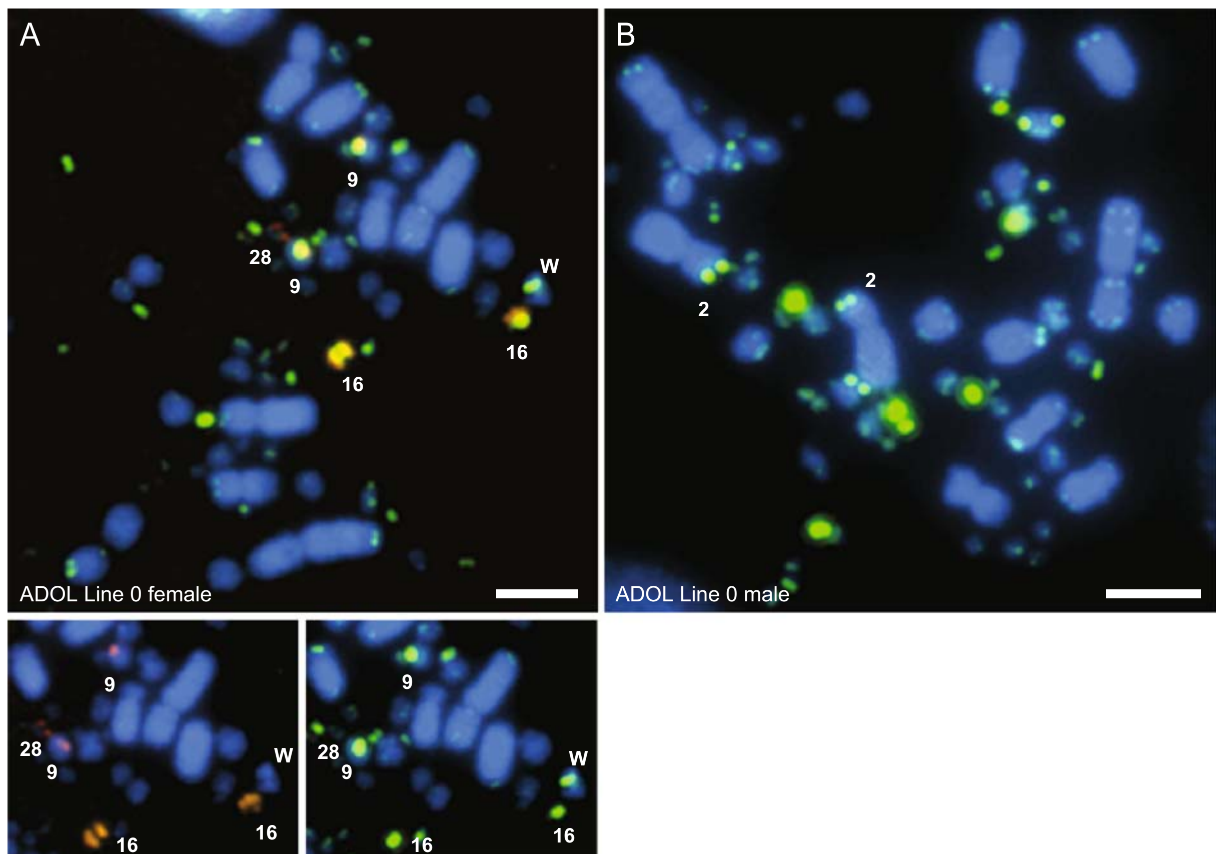


Fig. 3 Chromosomal locations of the mega-telomeres in ADOL Line 0. **A** Female cell with mega-telomeres shown on both homologs of GGA 9 and 16, and GGA W. GGA 28 was also tested and found to be negative for a mega-telomere. Although only one GGA 28 probe signal is present in this cell, two signals were seen in other cells. The *insets* below the female cell (**A**) show the chromosomal-specific probe signals for GGA 9 (*red*, 5S rDNA), 16 (*orange*, external transcribed spacer rDNA), and 28 (*red*, TAM32-4G3 BAC) on the left and the telomere-PNA probe signal (*green*) on the right. **B** Male cell shows a mega-telomere on both GGA 2 homologs which was not observed in the female sample. All images were adjusted to show brightest telomeres (see "Materials and methods"). *Scale bar*, 5 μm

complete copy as part of the derivative chromosome because two BACs known to identify major histocompatibility complex (MHC)-B and MHC-Y (Table 1) mapped to the same chromosome as the ETS ribosomal DNA probe. Both Figs. 4 and 5 show a GC-rich (DAPI light) region associated with GGA 16.

Telomeric array sizing of UCD 001, DT40, and DF-1

A molecular approach was used to further characterize telomeric array lengths within and among the three genotypes, UCD 001, DT40, and DF-1. Class I interstitial telomeric arrays (1–10 Kb) were studied. All three genotypes were shown to have a pattern consistent with interstitial telomeric arrays in terms of size and a discrete banding pattern (Fig. 6A). The condition employed to resolve Class I telomeric arrays also provided an overall view of the telomere length profile, i.e., Class II arrays (10–40 Kb) with some insight into Class III (>50 Kb). While both UCD 001 and DF-1 (Fig. 6A lanes 1 and 3, respectively) have significant telomeric array hybridization present at sizes of 20 Kb and above, DT40 (Fig. 6A, lane 2) lacked evidence for telomeric array sizes above approximately 20 Kb.

The genotypes were analyzed for Class III (>50 Kb) telomeric array profiles using three different PFGE conditions (see "Materials and methods"), which have different resolving power for the larger fragments. The PFGE condition 1 (Fig. 6B), which

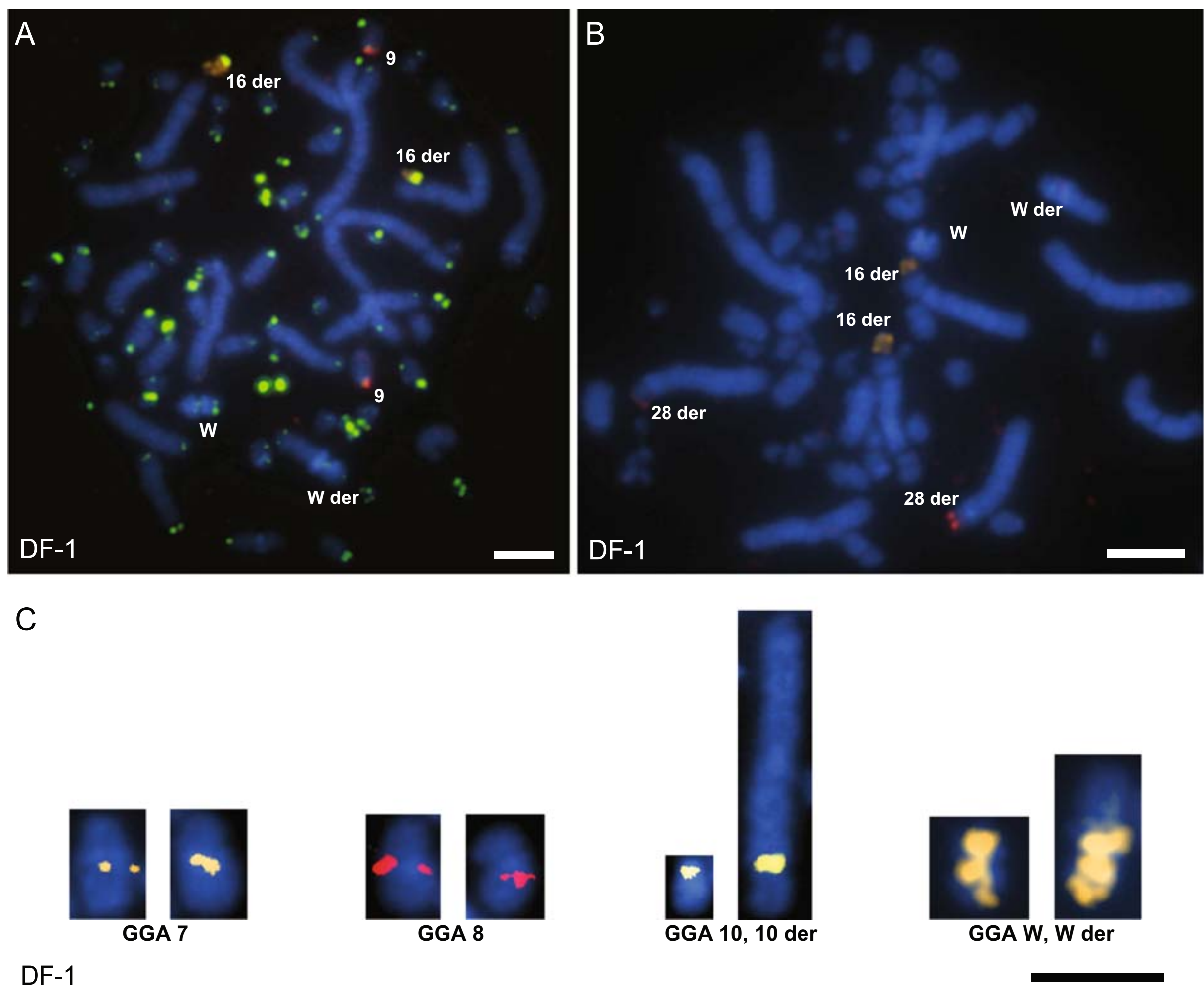


Fig. 4 The DF-1 immortalized cell line has a "derivative" karyotype with chromosomal fusions. Chromosomes 7, 8, 9, 10, 16, 28, and W were analyzed. **A** Both GGA 9 homologs (*red* signal, 5S rDNA) appear normal by size and probe specificity. GGA 9 does not possess a mega-telomere. Both GGA 16 homologs (*orange* signal, external transcribed spacer rDNA) are part of a derivative chromosome (16 der) and display a mega-telomere at one terminus of the derivative chromosome. GGA W is present in two copies, one normal in terms of size and DAPI-staining pattern and one derivative (fused to another chromosome, W der). **B** Both GGA 28 homologs (*red* signal, TAM32-4G3 BAC) are part of a derivative chromosome (28 der). **C** The chromosomes shown display chromosome-specific probe signals (see Table 1). Both GGA 7 and GGA 8 homologs appear normal by size and architecture. GGA 10 appears to exist in a heterozygous condition for a fusion (derivative chromosome), one homolog appears normal in size and architecture, whereas the other homolog is fused to a larger chromosome (10 der). Interestingly, two copies of GGA W are present in DF-1 cells. A GGA W specific probe labeled both the normal-sized W and W der. *Scale bar*, 5 µm

resolves telomeric arrays in the range of 50 to 800 Kb indicated that all of the UCD 001 individuals ($n=8$) analyzed possessed telomeric arrays at or above 1 Mb. The females exhibited two discrete telomeric arrays (Fig. 6B, lanes 1 and 2), and the males displayed one telomeric array (Fig. 6B, lanes 3 and 4). Further analysis was conducted by employing PFGE condition 3 (Fig. 6C), which resolves telomeric array sizes of 1 to 3 Mb. This condition allowed for the sizing of the telomeric arrays present in both males and females at 1.2 Mb (Fig. 6C, lanes 1–4). However, the female-specific array required further resolution because it was above 3.1 Mb (Fig. 6C, lanes 1 and 2) wherein PFGE condition 4 was able to resolve the UCD 001 female-specific array as approximately 4 Mb (Fig. 6D, lanes 1 and 2). Utilizing PFGE condition 1, DT40 showed four

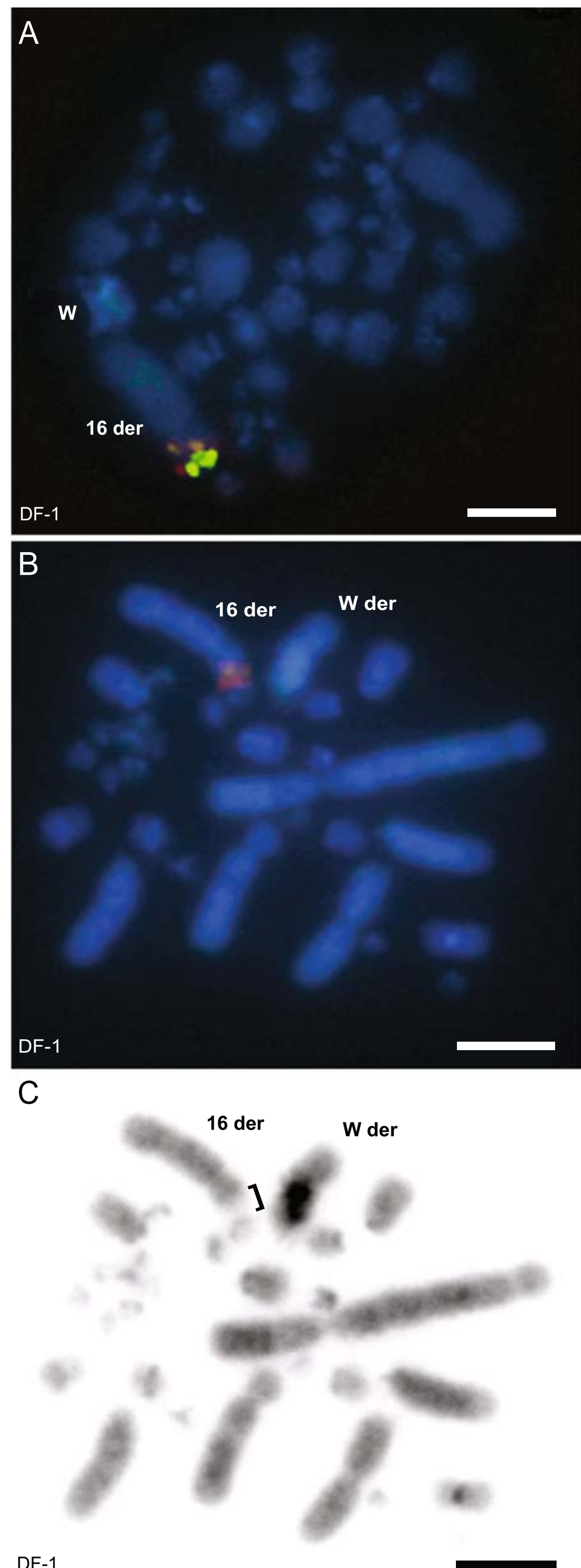


Fig. 5 The DF-1 GGA 16 derivative chromosomes contain an intact GGA 16 fused at its q terminus to another chromosome. GGA 16 encodes the nucleolus organizer region (NOR, the18S-5.8S-28S rRNA gene repeats) and the two major histocompatibility loci (MHC-B and MHC-Y). Multi-color FISH using probes specific for each genic region were utilized to assess the status and organizational features of GGA 16 in the DF-1 karyotype. In addition, this figure highlights the ploidy variability of DF-1. The cells shown are haploid; 14% of the cells in DF-1 cultures are haploid. **A** DF-1 haploid cell showing all three GGA 16 gene complex regions are present on the fused, derivative chromosome (16 der) and with a gene order as reported (Delany et al. 2009): NOR (*green*), MHC-Y (*red*), and MHC-B (*orange*). In this haploid cell, a normal-sized GGA W is present. **B** A different haploid cell shows MHC-Y (*red* signal) and MHC-B (*orange* signal) positioned across the DAPI-dull (GC rich) region on GGA 16. This haploid cell contains the W der chromosome. **C** Inverse image of the cell shown in (**B**) illustrates the GC rich (DAPI-dull) region on GGA 16 separating the NOR/MHC-Y from the MHC-B. *Scale bar*, 5 µm

discrete bands (<48, approximately 70, 100, and 500 Kb; Fig. 6B, lane 5), while DF-1 indicated numerous bands (>10) in this telomeric array size range from approximately 48 Kb to 1 Mb (Fig. 6B, lane 6).

Total telomeric sequence content: UCD 001, DT40, and DF-1

The total amount of telomeric sequence per genome (inclusive of interstitial and terminal arrays) was determined using a slot blot method of analysis (Fig. 7). An equivalent amount of DNA (100 ng) was analyzed in triplicate for each genotype, and the results were averaged between two slot blot experiments. The DF-1 cell line was found to contain 17%, UCD 001 indicated 5%, and DT40 had only 1.2% telomeric sequence per genome.

Discussion

Telomeric array variation exists within and among chicken genotypes

This study analyzed telomeric array variation at the intra-genomic (among chromosomes within a genome), inter-individual (among individuals within a genotype), and inter-genotype (among genotypes) levels considering array lengths, mega-telomere map locations, and total telomeric sequence content. Along with cells of

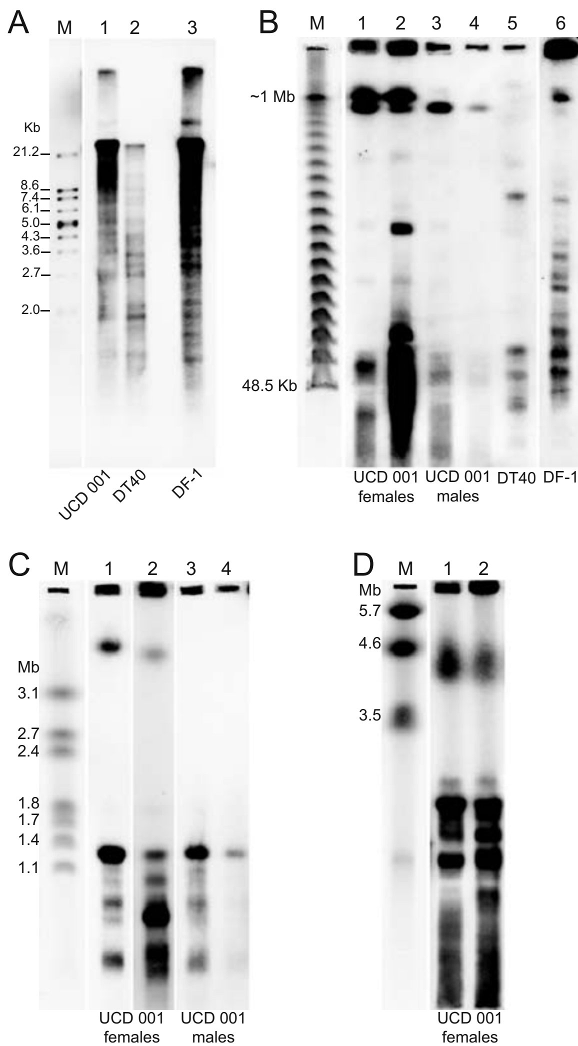
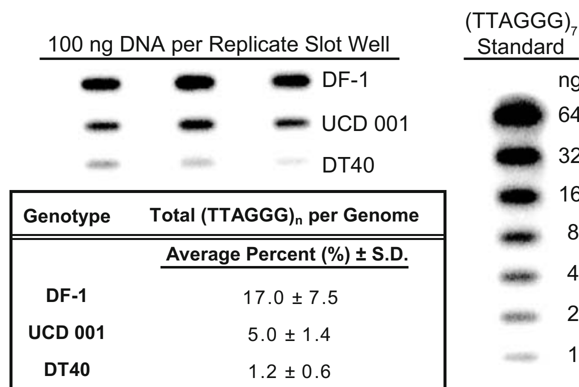


Fig. 6 Molecular sizing of telomeric array lengths of three chicken genotypes (UCD 001, DT40, and DF-1) by electrophoretic separation and Southern blotting with a telomeric sequence probe. A sex-specific telomeric array in females and genotype diversity for the mega-telomere arrays are illustrated. **A** Standard electrophoresis separates arrays in the size range of 0.5–20 Kb, which includes interstitial arrays (Delany et al. 2000) and are found in all the genotypes studied. The standard separation also illustrates UCD 001 and DF-1 have telomeric arrays present above 21 Kb (*lanes 1* and *3*, respectively), while DT40 does not (*lane 2*). Equivalent amounts of DNA (150 ng) were utilized; however, DF-1 hybridization was the darkest (*lane 3*), and DT40 was the lightest (*lane 2*) for overall telomeric sequence hybridization. **B** PFGE condition 1 resolves terminal arrays in the size range of 50–800 Kb. The DF-1 genome has many arrays in this size range (>10, see *lane 6*), whereas UCD 001 females have two large telomeric arrays and UCD 001 males have one telomeric array present at approximately 1 Mb (see *lanes 1–4*). The marker is a Lambda Ladder PFG Marker (New England Biolabs, N0340S) concatemer ladder (48.5 Kb increments). **C** PFGE condition 3 resolves terminal arrays in the size range of 1–3 Mb. UCD 001 males and females share a telomeric array in common of approximately 1.2 Mb in length, whereas the females have an additional telomeric array above 3.1 Mb. The marker is *Hansenula wingei* chromosomes (Bio-Rad, 170-3667). **D** PFGE condition 4 resolves terminal arrays in the size range of 3.5–5.7 Mb. The UCD 001 female-specific array (shown in **c** to be above 3.1 Mb) is between 3.5 and 4.6 Mb, approximately sizing to 4.0 Mb. The marker is *Schizosaccharomyces pombe* chromosomal DNA (Bio-Rad, 170-3633). *Lanes 1 and 2* on (**B**), (**C**), and (**D**) are the same females; *lane 2* on (**C**) is from a separate gel than the other lanes and therefore the upper band migrated to a slightly different degree. *Lanes 3 and 4* on (**B**) and (**C**) are the same male samples (which are the CEF males used in the FISH experiments)

normal phenotype, cells were incorporated in the analysis exhibiting immortalized and transformed phenotypes. The objective was to consider telomeric array variation in the context of cellular proliferation "potential" because the maintenance of telomeric array size is integral to senescence, aging, and transformation events (Shay and Wright 2005; Swanberg and Delany 2006; Deng and Chang 2007). Both molecular and cytogenetic approaches were utilized to gain an integrated view of the variation, from the DNA to chromosomal level. Knowledge of telomeric array variation in different biological systems (having different genotypes and phenotypes) should contribute to exploring the mechanisms of telomere regulation and maintenance in the model chicken vertebrate system.

Chicken genetic lines

To date, three different inbred chicken genetic lines have been analyzed for mega-telomere status, two of those are of the Single Comb White Leghorn (SCWL) breed (UCD 003, Delany et al. 2007; ADOL Line 0, this study) and one is a Red Jungle Fowl (RJF) line (UCD 001, this study). The RJF are considered the monophyletic ancestor to the domesticated breeds (Fumihito et al. 1994), and one female from the UCD 001 RJF line served as the sequenced chicken genome (ICGSC 2004). Among the various genetic lines, both in-common mega-telomere loci were mapped (GGA 9 and W) as well as unique loci (see Table 1). The SCWL lines UCD 003 and ADOL Line 0 exhibited a greater number of mega-telomere loci than the RJF UCD 001. Interestingly, such dynamic variations have also been reported in the murine system. The domesticated



Genotype	Total $(TTAGGG)_n$ per Genome
	Average Percent (%) ± S.D.
DF-1	17.0 ± 7.5
UCD 001	5.0 ± 1.4
DT40	1.2 ± 0.6

Fig. 7 Total telomeric sequence content varies significantly among chicken genotypes: DF-1, UCD 001, and DT40. The total telomeric sequence, which is inclusive of interstitial plus terminal arrays, was determined on a per genome basis for each genotype by slot blot procedures and analysis. One hundred nanograms of DNA was loaded in each slot blot well for each sample in triplicate. A telomeric G-rich strand oligonucleotide $(TTAGGG)_7$ was used as a standard in concentrations from 1 to 64 ng. The percentage of telomeric sequence for each genotype was calculated by averaging the triplicates of two separate slot blot experiments. Total telomeric sequence content in DF-1 was calculated to be 17%, in UCD 001 was 5%, and in DT40 was 1.2% per genome

laboratory mouse (*M. musculus*) genome contains ultra-long telomeres, while the wild mouse (*M. spretus*) genome does not (Hemann and Greider 2000); inbreeding of the white-footed mouse was shown to result in increases of telomeric array length (Manning et al. 2002). In the chicken, unlike the mouse, significant intra-genomic variation of telomere length exists, i.e., not all chromosome ends are of mega-telomere status (Rodrigue et al. 2005; Delany et al. 2007, this study). Mega-telomere arrays and intra-genomic variation are a feature of a number of bird lineages, although not all (Delany et al. 2000; Nanda et al. 2002). The presence of mega-telomeres (albeit fewer) in the RJF UCD 001 line is suggestive that such arrays were present before domestication and that perhaps selective breeding contributes to generation of more loci with longer arrays in the chicken (e.g., as seen in inbred UCD 003 and the closed ADOL Line 0). The value or function of the mega-telomere for avian genomes remains unclear.

Two chromosomes that exhibit mega-telomeres among all genetic lines include GGA 9 (at the p arm end) and GGA W (q arm end). These chromosomes are similar in size to each other (W is the tenth largest chromosome), and both encode repetitive elements. GGA 9 encodes the 5S ribosomal DNA repeat (near the mega-telomere), and interestingly, the telomerase RNA gene maps to the q arm of 9 (Daniels and Delany 2003; Delany and Daniels 2003). The W consists almost entirely of repeat sequences with only a few known genes (Schmid et al. 2000, 2005; ICGSC 2004).

The GGA W mega-telomere was sized for both UCD 003 and UCD 001, 2.8 Mb (Rodrigue et al. 2005) and approximately 4 Mb (this study), respectively. The difference of approximately 1 Mb was not obvious by cytogenetic analysis, but clearly GGA W displays the brightest telomere signal of all chromosomes within both genetic lines. The molecular telomeric array size difference could be due to copy number variation of telomeric repeats and/or caused by loss or gain of one or more HaeIII restriction enzyme sites changing the amount of flanking DNA. The UCD 001 GGA 9 mega-telomere array was found to be 1.2 Mb. The sizing of GGA 9 was possible because cytogenetically females have two mega-telomere loci (GGA 9 and W), whereas males only have one locus (GGA 9), and by molecular sizing, females have two ultra-long telomeric arrays (1.2 and 4 Mb), whereas males have only one telomeric array at 1.2 Mb.

Immortalized and transformed chicken cell lines

The cell lines indicated unexpected profiles relative to each other and the in vivo resources. The immortalized DF-1 cell line by far has the most telomeric sequence content, as shown by cytogenetic (FISH) and molecular sizing analyses, and the DF-1 profile was very different from that seen in the transformed cell line, DT40, which gave no indication of mega-telomeres. To integrate the results, total telomeric sequence content was established to quantify on a genome basis the results as seen by the other techniques. As predicted from the other assays, DF-1 has the most telomeric sequence content, 17%, which is more than three times the amount in UCD 001 (5%). The DT40 genome exhibited the lowest amount of telomeric sequence, 1.2%, more than tenfold less than DF-1 and about fourfold less than UCD 001. Previously, it was determined that normal chicken lines exhibited 3–4% telomeric sequence content per genome (Delany et al. 2000). These are surprising results because DT40 is known to be

highly telomerase-positive (Swanberg and Delany 2003) while DF-1 is reported to be telomerase-negative (Christman et al. 2005). Both of these cell lines are well utilized by the avian and vertebrate research communities for a variety of molecular and biomedical studies, and thus, understating their telomere biology has ramifications for usage of these cell types for other analyses.

Although Cooley et al. (2009) report DT40 telomere lengths to be 17–43 Kb for the macrochromosomes and 70 Kb–1 Mb for the microchromosomes, our combined cytogenetic and molecular results suggest a different profile. In our study, arrays of large size (e.g., Fig. 6B, lane 5) were observed; however, these had overall low hybridization signals suggesting the large size might be due to flanking DNA versus telomeric sequence. Our cytogenetic results show relatively less intense signals (as compared to the other genomes run in the same FISH experiments) which could not be enhanced and were reproducible in replicate experiments. These results combined with the slot blot analysis showing DT40 to have fourfold less telomeric sequence content than UCD 001 suggest this genome is unusual for its low telomeric sequence content.

A recent study reported lack of success in deleting both copies of the telomerase RNA (TR) gene in DT40, and the deletion of one copy reduced proliferation, suggesting that TR is necessary for DT40 survival (Faure et al. 2008). Expression of the telomerase genes, TR, and telomerase reverse transcriptase (TERT) is upregulated in DT40 as compared to highly expressing gastrula stage embryos (Swanberg and Delany 2005, O'Hare and Delany 2005) and chicken embryonic stem cells (Swanberg et al. 2004). The chromosome (GGA 2) encoding TERT (Delany and Daniels 2004) is trisomic in DT40 (Sonoda et al. 1998; Chang and Delany 2004). Upregulation of the telomerase genes could be a requirement to provide an enhanced level of telomerase activity to maintain critically shortened telomeres, as in human tumor cells (Counter et al. 1994). Reduced proliferation resulting from TR gene deletion (Faure et al. 2008), extensive evidence for TR and TERT expression upregulation despite a low telomeric sequence content, and the striking lack of long telomeres as seen in other chicken cells suggest a model wherein a stringent requirement exists for telomerase because of the short telomeres being at the "threshold" levels for survival.

The DF-1 cell line originated from a primary CEF cell culture (established from ADOL Line 0 embryos) which senesced and then emerged from crisis as immortalized (Himly et al. 1998). Primary CEFs are known to be telomerase-negative and exhibit shortened telomeres over continued population doublings (Swanberg and Delany 2003). The DF-1 cell line could provide a useful model system to explore non-telomerase based mechanisms for maintenance of telomeres as our results show a telomere profile with many large telomeric arrays despite being telomerase-negative (Christman et al. 2005). Along with the unexpected telomere profile, DF-1 is also composed of a complex derivative karyotype containing numerous chromosomal fusions both in the homozygous and heterozygous condition. For the derivative chromosomes characterized in this study, fusion orientation was evaluated, e.g., p arm fused to another chromosome, and at the fusion sites, no interstitial telomeric array signals were apparent. A model which explains the observations from this study and prior research is that the original primary CEF culture developed critically shortened telomeres as would be expected resulting in chromosomal fusions and karyotype changes. During the immortalization process the DF-1 cell line developed alternative mechanisms than the telomerase pathway to maintain and lengthen its telomeres. In this regard, one of the reasons ADOL Line 0 was incorporated into this study was because DF-1 was derived from pooled embryos of this genetic line, allowing for a general comparison of features. It is clear via cytogenetic analysis that DF-1 has an enhanced telomeric array profile relative to ADOL Line 0 along with the significantly abnormal karyotype.

A mega-telomere locus was mapped to the p arm of GGA 16 in DF-1 (an in-common site with the UCD 003 and ADOL Line 0 genomes), and thus it was determined that GGA 16 fused via its q terminus to another chromosome. The chromosome 16 portion of the derivative chromosome appears intact, since previously mapped gene complexes on GGA 16 were identified by FISH and in the appropriate order: NOR/MHC-Y and MHC-B (Delany et al. 2009). An example of a derivative chromosome in the heterozygous condition is GGA 10. Based on size and probe position, one GGA 10 fused at its p terminus to another chromosome. The analyzed chromosomes that appeared normal in DF-1 include GGA 7, 8, 9, and one GGA 10 homolog. The status of these

chromosomes was determined by size, morphology, and probe specificity. The probe position was analyzed cytogenetically (chromosome ends, p vs q arm, near centromere, etc.) and then aligned with genome data obtained from UCSC Genome Browser (http://genome.ucsc.edu) Chicken May 2006 assembly. For example, GGA 7 assembled sequence is 38.4 Mb, and the BAC-probe (CH261-95H15) position is 19.7 Mb (roughly center); GGA 8 assembled sequence is 30.7 Mb, and probe (CH261-84K8) position is 15.7 Mb (roughly center); GGA 9 assembled sequence is 25.6 Mb, and 5S is approximately at 1.9 Mb (therefore, p arm proximal); and GGA 10 assembled sequence is 22.6 Mb and probe (TAM33-42N22) position is 1.3 Mb (p arm). Chromosome 1 was identified easily by its morphological features and interstitial telomeric array pattern, which is similar in DF-1 as compared to the other genotypes. However, GGA 1 appears to be present in only one copy (diploid cells) and to have a chromosomal fusion at the q arm, with a secondary constriction. Most surprisingly, the female-specific sex chromosome W is present in two copies, one derivative (fused to another chromosome by its p terminus) and one normal. In the triploid chicken model, ZWW (a double dose of GGA W) is lethal in embryos (Thorne et al. 1991; Thorne and Sheldon 1993). Thus, it has been presumed that the W contains elements that are dosage-dependent lethal.

Conclusions

This study provides evidence that GGA 9p and Wq maintain mega-telomere arrays among a diversity of chicken genetic lines, and that other such loci are variable among genotypes and to varying degrees, e.g., GGA 16. The female-specific W chromosome mega-telomere is the largest observed by FISH and was sized in UCD 001 as approximately 4.0 Mb and in UCD 003 as 2.8 Mb. Cytogenetic and molecular analyses suggest that both DT40 and DF-1 cells appear to have an altered telomere profile relative to normal cells suggestive of dysregulation of the telomere-telomerase pathways. The karyotype fusions evident in DF-1 are interesting in this regard. Further analysis is required to more fully understand telomere maintenance mechanisms in immortalized and transformed avian cells and understand the role of the mega-telomeres in the avian genome.

Acknowledgements The authors thank Drs. Marcia Miller for donation of MHC-B and -Y probes and Jerry Dodgson for advice regarding BAC insert size calculations. The DT40 cells were a gift from Dr. Jean-Marie Buerstedde. This project was supported by National Research Initiative Grant no. 2005-35205-16679 from the USDA Cooperative State Research, Education, and Extension Service (CSREES) Animal Genome program and USDA-CSREES Multistate Research Projects NE-1016 (CA-D*-ASC-7281-RR) and NRSP-8 (CA-D*-ASC-7233-RR). We gratefully acknowledge the UC Davis College of Agricultural and Environmental Sciences and the California Agricultural Experiment Station for infrastructure support for the poultry genetic resources used in this study. Student fellowship support was provided by the Austin Eugene Lyons Fellowship and the Department of Animal Science. We appreciate the helpful comments of the anonymous reviewers.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

Abplanalp H (1992) Inbred lines as genetic resources of chickens. Poult Sci Rev 4:29–39

Baba TW, Giroir BP, Humphries EH (1985) Cell lines derived from avian lymphomas exhibit two distinct phenotypes. Virology 144:139–151

Bacon LD, Hunt HD, Cheng HH (2000) A review of the development of chicken lines to resolve genes determining resistance to diseases. Poult Sci 79:1082–1093

Blasco MA (2008) Telomere binding proteins and disease. In: Rudolph KL (ed) Telomeres and telomerase in ageing, disease, and cancer. Springer-Verlag, Berlin, pp 229–244

Bolzán AD, Bianchi MS (2006) Telomeres, interstitial telomeric repeat sequences, and chromosomal aberrations. Mutat Res 612:189–214

Chang H, Delany ME (2004) Karyotype stability of the DT40 chicken B cell line: macrochromosome variation and cytogenetic mosaicism. Chromosome Res 12:299–307

Christman SA, Kong BW, Landry MM, Kim H, Foster DN (2005) Modulation of p53 expression and its role in the conversion to a fully immortalized chicken embryo fibroblast line. FEBS Lett 579:6705–6715

Cooley C, Baird KM, Faure V, Wenner T, Stewart JL, Modino S, Slijepcevic P, Farr CJ, Morrison CG (2009) Trf1 is not required for proliferation or functional telomere maintenance in chicken DT40 cells. Mol Biol Cell 20:2563–2571

Counter CM, Hirte HW, Bacchetti S, Harley CB (1994) Telomerase activity in human ovarian carcinoma. Proc Natl Acad Sci 91:2900–2904

Coviello-McLaughlin GM, Prowse KR (1997) Telomere length regulation during postnatal development and ageing in *Mus spretus*. Nucleic Acids Res 25:3051–3058

Dahse R, Fiedler W, Ernst G (1997) Telomeres and telomerase: biological and clinical importance. Clin Chem 43:708–714

Daniels LM, Delany ME (2003) Molecular and cytogenetic organization of 5S ribosomal DNA array in chicken (*Gallus gallus*). Chromosome Res 11:305–317

Davis T, Kipling D (2005) Telomeres and telomerase biology in vertebrates: progress towards a non-human model for replicative senescence and ageing. Biogerontology 6:371–385

Delany ME, Pisenti JM (1998) Conservation of poultry genetic research resources: considerations of the past, present, and further. Poult Avian Biol Rev 9:25–42

Delany ME, Krupkin AB (1999) Molecular characterization of ribosomal gene variation within and among NORs segregating in specialized populations of chicken. Genome 42:60–71

Delany ME, Krupkin AB, Miller MM (2000) Organization of telomere sequences in birds: evidence for arrays of extreme length and for in vivo shortening. Cytogenet Cell Genet 90:139–145

Delany ME, Daniels LM (2003) The chicken telomerase RNA gene: conservation of sequence, regulatory elements and synteny among viral, avian and mammalian genomes. Cytogenet Genome Res 102:309–317

Delany ME, Daniels LM (2004) The chicken telomerase reverse transcriptase (chTERT): molecular and cytogenetic characterization with a comparative analysis. Gene 339: 61–69

Delany ME, Daniels LM, Swanberg SE, Taylor HA (2003) Telomeres in the chicken: genome stability and chromosome ends. Poult Sci 82:917–926

Delany ME, Gessaro TM, Rodrigue KL, Daniels LM (2007) Chromosomal mapping of chicken mega-telomere arrays to GGA9, 16, 28 and W using a cytogenomic approach. Cytogenet Genome Res 117:54–63

Delany ME, Robinson CM, Goto RM, Miller MM (2009) Architecture and organization of chicken microchromosome 16: order of the NOR, MHC-Y and MHC-B subregions. J Heredity 100:507–514

Deng Y, Chang S (2007) Role of telomeres and telomerase in genomic instability, senescence and cancer. Lab Invest 87:1071–1076

Faure V, Wenner T, Cooley C, Bourke E, Farr CJ, Takeda S, Morrison CG (2008) Ku70 prevents genome instability resulting from heterozygosity of the telomerase RNA component in vertebrate tumour line. DNA Repair (Amst) 7:713–724

Fumihito A, Miyake T, Sumi S, Takada M, Ohno S, Kondo N (1994) One subspecies of the red junglefowl (*Gallus gallus gallus*) suffices as the matriarchic ancestor of all domestic breeds. Proc Natl Acad Sci 91:12505–12509

Hartmann N, Scherthan H (2004) Characterization of ancestral chromosome fusion points in the Indian muntjac deer. Chromosoma 112:213–220

Hemann MT, Greider CW (2000) Wild-derived inbred mouse strains have short telomeres. Nucleic Acids Res 28:4474–4478

Hemann MT, Strong MA, Hao LY, Greider CW (2001) The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. Cell 107:67–77

Henson JD, Neumann AA, Yeager TR, Reddel RR (2002) Alternative lengthening of telomeres in mammalian cells. Oncogene 21:598–610

Himly M, Foster DN, Bottoli I, Iacovoni JS, Vogt PK (1998) The DF-1 chicken fibroblast cell line: transformation induced by diverse oncogenes and cell death resulting from infection by avian leukosis viruses. Virology 248:295–304

International Chicken Genome Sequencing Consortium (ICGSC) (2004) Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. Nature 432:695–716

Kim S, Parrinello S, Kim J, Campisi J (2003) *Mus musculus* and *Mus spretus* homologues of the human telomere-associated protein TIN2. Genomics 81:422–432

Kipling D, Cooke HJ (1990) Hypervariable ultra-long telomeres in mice. Nature 347:400–402

Lee C, Sasi R, Lin CC (1993) Interstitial localization of telomeric DNA sequences in the Indian muntjac chromosomes: further evidence for tandem chromosome fusions in the karyotypic evolution of the Asian muntjacs. Cytogenet Cell Genet 63:156–159

Lee MK, Ren CW, Yan B, Cox B, Zhang HB, Romanov MN, Sizemore FG, Suchyta SP, Peters E, Dodgson JB (2003) Construction and characterization of three BAC libraries for analysis of the chicken genome. Anim Genet 34:151–152

Manning EL, Crossland J, Dewey MJ, Van Zant G (2002) Influences of inbreeding and genetics on telomere length in mice. Mamm Genome 13:234–238

McClintock B (1941) The stability of broken ends of the chromosome in *Zea mays*. Genetics 26:234–282

Muller HJ (1938) The remaking of chromosomes. Collecting Net 13:181–198

Nanda I, Schmid M (1994) Localization of the telomeric $(TTAGGG)_n$ sequence in chicken (*Gallus domesticus*) chromosomes. Cytogenet Cell Genet 65:190–193

Nanda I, Schrama D, Feichtinger W, Haaf T, Schartl M, Schmid M (2002) Distribution of telomeric $(TTAGGG)_n$ sequences in avian chromosomes. Chromosoma 111:215–227

Ogawa A, Solovei I, Hutchison N, Saitoh Y, Ikeda J, Macgregor H, Mizuno S (1997) Molecular characterization and cytological mapping of non-repetitive DNA sequence region from the W chromosome of chicken and its use as a universal probe for sexing Carinatae birds. Chromosome Res 5:93–101

O'Hare TH, Delany ME (2005) Telomerase gene expression in the chicken: Telomerase RNA (TR) and reverse transcriptase (TERT) transcript profiles are tissue-specific and correlate with telomerase activity. AGE 27:257–266

Pitel F, Pouzadoux A, Langlois P, Fillon V, Heimel C, Douaire M, Gellin J, Vignal A (1998) Mapping of the chicken *SCD1* locus: assignment of a linkage group to chromosome 6. Anim Genet 29:152

Prowse KR, Greider CW (1995) Development and tissue-specific regulation of mouse telomerase and telomere length. Proc Natl Acad Sci 92:4818–4822

Reddel RR, Bryan TM, Colgin LM, Perrem KT, Yeager TR (2001) Alternative lengthening of telomeres in human cells. Radiat Res 155:194–200

Ren C, Lee MK, Yan B, Ding K, Cox B, Romanov MN, Price JA, Dodgson JB, Zhang HB (2003) A BAC-based physical map of the chicken genome. Genome Res 13:2754–2758

Rodionov AV, Lukina NA, Galkina SA, Solovei I, Saccone S (2002) Crossing over in chicken oogenesis: cytological and chiasma-based genetic maps of the chicken lampbrush chromosome 1. J Heredity 93:125–129

Rodrigue KL, May BP, Famula TR, Delany ME (2005) Meiotic instability of chicken ultra-long telomeres and mapping of a 2.8 megabase array to the W-sex chromosome. Chromosome Res 13:581–591

Royle NJ, Foxon J, Jeyapalan JN, Mendez-Bermudez A, Novo CL, Williams J, Cotton VE (2008) Telomere length maintenance—an ALTernative mechanism. Cytogenet Genome Res 122:281–291

Scheel C, Poremba C (2002) Telomere lengthening in telomerase-negative cells: the ends are coming together. Virchows Arch 440:573–582

Schmid M, Nanda I, Guttenbach M, Steinlein C, Hoehn H, Schartl M, Haaf T, Weigend D, Fries R, Buerstedde JM, Wimmers K, Burt DW, Smith J, A'Hara S, Law A, Griffin DK, Bumstead N, Kaufman J, Thomson PA, Burke TA, Groenen MAM, Crooijmans RPMA, Vignal A, Fillion V, Morisson M, Pitel F, Tixier-Boichard M, Ladjali-Mohammedi K, Hillel J, Mäki-Tanila A, Cheng HH, Delany ME, Burnside J, Mizuno S (2000) First report on chicken genes and chromosomes. Cytogenet Cell Genet 90:169–218

Schmid M, Nanda I, Hoehn H, Schartl M, Haaf T, Buerstedde JM, Arakawa H, Caldwell RB, Weigend S, Burt DW, Smith J, Griffin DK, Masabanda J, Groenen MAM, Crooijmans RPMA, Vignal A, Fillon V, Morisson M, Pitel F, Vignoles M, Garrigues A, Gellin J, Rodionov AV, Galkina SA, Lukina NA, Ben-Ari G, Blum S, Hillel J, Twito T, Lavi U, David L, Feldman MW, Delany ME, Conley CC, Fowler VM, Hedges SB, Godbout R, Katyal S, Smith C, Hudson Q, Sinclair A, Mizuno S (2005) Second report on chicken genes and chromosomes. Cytogenet Genome Res 109:415–479

Shawi M, Autexier C (2008) Telomerase, senescence and ageing. Mech Ageing Dev 129:3–10

Shay JW, Wright WE (2005) Senescence and immortalization: role of telomeres and telomerase. Carcinogenesis 26:867–874

Shiina T, Briles WE, Goto RM, Hosomichi K, Yanagiya K, Shimizu S, Inoko H, Miller MM (2007) Extended gene map reveals tripartite motif, C-type lectin, and Ig superfamily type genes within a subregion of the chicken *MHC-B* affecting infectious disease. J Immunol 178:7162–7172

Sonoda E, Sasaki MS, Buerstedde JM, Bezzubova O, Shinohara A, Ogawa H, Takata M, Yamaguchi-Iwai Y, Takeda S (1998) Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death. EMBO J 17:598–608

Swanberg SE, Delany ME (2003) Dynamics of telomere erosion in transformed and non-transformed avian cells in vitro. Cytogenet Genome Res 102:318–325

Swanberg SE, Delany ME (2005) Differential expression of genes associated with telomere length homeostasis and oncogenesis in an avian model. Mech Ageing Dev 126:1060–1070

Swanberg SE, Delany ME (2006) Telomeres in aging: birds. In: Conn PM (ed) Handbook of models for human aging. Elsevier Academic Press, Burlington, pp 339–349

Swanberg SE, Payne WS, Hunt HD, Dodgson JB, Delany ME (2004) Telomerase activity and differential expression of telomerase genes and c-myc in chicken cells in vitro. Dev Dyn 231:14–21

Taylor HA, Delany ME (2000) Ontogeny of telomerase in chicken: impact of downregulation on pre- and post-natal telomere length in vivo. Dev Growth Differ 42:613–621

Thorne MH, Sheldon BL (1993) Triploid intersex and chimeric chickens: useful models for studies of avian sex determination. In: Reed KC, Graves JAM (eds) Sex chromosomes and sex determining genes. Harwood Academic Publishers, Switzerland, pp 199–205

Thorne MH, Collins RK, Sheldon BL (1991) Triploidy and other chromosomal abnormalities in a selected line of chickens. Genet Sel Evol 23:212s–216s

Wells RA, Germino GG, Krishna S, Buckle VJ, Reeders ST (1990) Telomere-related sequences at interstitial sites in the human genome. Genomics 8:699–704