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Meiotic instability of chicken ultra-long telomeres and mapping of a 2.8 megabase array to the W-sex chromosome

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Abstract

The objective of this research was to study the meiotic stability of a subset of chicken telomere arrays, which are the largest reported for any vertebrate species. Inheritance of these ultra-long telomere arrays (200 kb to 3 Mb) was studied in a highly homozygous inbred line, UCD 003 ($F > 0.99$). Analysis of array transmission in four families indicated unexpected heterogeneity and non-Mendelian segregation including high-frequency-generation of novel arrays. Additionally, the largest array detected (2.8 Mb) was female-specific and correlated to the most intense telomeric DNA signal on the W-sex chromosome by fluorescence *in situ* hybridization (FISH). These results are discussed in regard to the potential functions of the ultra-long telomere arrays in the chicken genome including generation of genetic variation through enhanced recombination, protection against erosion by providing a buffer for gene-dense regions, and sex-chromosome organization.

Introduction

Vertebrate telomeres consist of the tandemly repeated DNA sequence 5′-TTAGGG-3′, complexed with DNA binding proteins. Telomeres provide a mechanism for chromosome end-replication and are involved in transcriptional silencing, chromosome pairing and segregation, and nuclear organization (Blackburn 1994, Shore 1995, Zakian 1995, Stavenhagen & Zakian 1998). Telomeres are replicated by the enzyme telomerase which adds telomeric sequence *de novo* to the termini of chromosomes (Greider & Blackburn 1989). Correlating with telomerase down-regulation in somatic cells, telomeres shorten in aging cells of both human and chicken (Henderson *et al*. 1996, Taylor & Delany 2000, Forsyth *et al*. 2002). Telomere shortening is an important genetic mechanism governing cellular lifespan and dys-regulation of the "telomere clock" is implicated in transformation and oncogenesis (Harley *et al*. 1990, Dhaene *et al*. 2000).

Chicken telomeric DNA arrays vary remarkably in size, position and stability; based on these features three classes are discerned (Delany *et al*. 2000, Taylor & Delany 2000, Delany *et al*. 2003). Class I telomeres are 0.5–10 kb, interstitial, do not shorten during aging, and are identical within inbred lines. Class II arrays are 10–40 kb, are located at the termini of chromosomes, and shorten in an age-related pattern *in vitro* and *in vivo*. Class III arrays are those larger than 40 kb, with most of the arrays in the 0.2–3 Mb range (termed ultra-long arrays); these arrays are terminal as shown by Bal31 digestion (Delany *et al*. 2000). The mitotic stability of Class III arrays is

uncharted. This is because although shortening could be predicted on the basis of Class II array erosion rates, e.g., 28–88 bases/population doubling *in vitro* (Swanberg & Delany 2003), and *in vivo* rates of 160 bases per hematopoietic cell division (Delany *et al*. 2000) or 600 bases/year in somatic tissues (Taylor & Delany 2000), such relatively small changes cannot be experimentally resolved for arrays 100s to 1000s of kb.

The first evidence for a class of extremely large arrays in the chicken genome was cytogenetic (Nanda & Schmid 1994). These arrays were suggested to be preferentially located on microchromosomes to provide protection against shortening for the smallest genetic elements of the genome (Delany *et al*. 2000). Nanda *et al*. (2002) hypothesized that these arrays contribute to the high recombination rate of chicken microchromosomes. Preliminary analysis of a small number of siblings from different highly inbred chicken lines suggested that the ultra-long arrays were extremely heterogeneous (Delany *et al*. 2000, 2003) contrasting markedly with genetic homogeneity for single, complex, and tandem repeat loci, e.g., microsatellite (Crittenden *et al*. 1993), MHC and other blood group antigens (Abplanalp 1992), telomerase RNA (Delany & Daniels 2003), and the rDNA complexes (Delany & Krupkin 1999, Daniels & Delany 2003). Interestingly, a category of large telomere arrays in the laboratory mouse (*Mus musculus*) show extensive heterogeneity, non-Mendelian inheritance and high-frequency generation of novel arrays (Starling *et al*. 1990).

Therefore, in this study the inheritance of chicken ultra-long telomere arrays was studied to investigate array transmission and germline stability. Only a very few arrays exhibited inheritance expected for an inbred line, and further, novel arrays were generated in progeny. The largest array was a 2.8 Mb female-specific array and correlated with the presence of a "mega"-telomeric DNA signal mapped by FISH to the W-chromosome.

Materials and methods

Chicken resources

This study utilized pedigreed animals from two generations of Single Comb White Leghorn chickens from the highly inbred UCD 003 line established in 1956 and maintained since by brother–sister matings (Abplanalp 1992, F > 0.99). A group of adults (n = 20) were screened for telomere array patterns and four full-sib mating pairs were established. Twenty-four progeny were collected for study per family. The progeny were sexed at collection by morphological examination of the gonads, as females show a significantly enlarged left gonad (Mittwoch 1998).

DNA preparation

Blood samples (0.3 ml) were collected in 0.2 ml anticoagulant (0.7 M NaCitrate/0.7 M NaCl) by venipuncture of the wing brachial vein of adults (parents) or by capillary action from the extra-embryonic membrane vessels of 13-day incubation embryos (progeny). Erythrocytes were counted using a hemacytometer and for each individual a dilution containing 3.2×10^6 cells (~8 μg DNA) was mixed 1:1 with 2% Pulsed Field Certified agarose (BioRad 162-0137) and heated to 65°C. The molten cell–agarose mixture was poured into a mold to create plugs with embedded cells (protocol followed according to BioRad CHEF-DR II Instruction Manual). Plugs were treated with cell lysis buffer (0.5 mM EDTA, 1 M Tris-HCl, 1% N-laurylsarcosine) and 0.5 mg proteinase K (Roche) overnight with rotation at 55°C. The plug-embedded DNA samples were digested using 4 μl *Hae* III (ProMega) overnight in a 35°C waterbath.

Telomere array analysis

Pulsed field gel electrophoresis. Briefly, DNA plugs were cut into equal-sized slices (number depending on the size of the mold) to have ~3 μg DNA per lane for size separation by pulsed-field gel electrophoresis (PFGE). The ultra-long telomeric DNA arrays were separated according to the following conditions: (1) 50–800 kb arrays were resolved using 0.8% agarose (see above), 30–70 s switch time, 5.2 V/cm and 0.5X TBE (44.5 mM Tris Base, 44.5 mM boric acid, 1 mM EDTA) for 20 h at 14°C; (2) arrays greater than 800 kb to 1 Mb were resolved using 1% agarose, 50–90 s switch time, 6 V/cm and 0.5X TBE buffer for 22 h at 14°C; (3) arrays to 3 Mb were resolved using 0.8% agarose, 250–900 s switch time, 3 V/cm and 1X TAE (Bio-Rad) for 50 h at 14°C. Electrophoresis conditions 1 and 2 always included the same male control sample and a λ ladder marker (50–800 kb). A

Hansenula wingei marker (Bio-Rad, 1–3 Mb) was used with condition 3. Gels were stained with ethidium bromide and examined for DNA content and lane uniformity.

Southern blot hybridizations. Gels were Southern blotted (Sambrook & Russell 2001) and hybridized with an α-^{32}P[dCTP]-end-labeled telomere probe $(5'\text{-TTAGGG-}3')_7$ according to standard procedures (Delany *et al*. 2000), except membranes were washed three times in 2× SSC/0.2% SDS for 15 min at 65°C prior to autoradiography. BioMax MR Autoradiograph Film (Eastman Kodak) was exposed using two intensifying screens for 15, 30, and 60 min, scanned, and analyzed with Kodak 1D 3.6 imaging software.

Analyses

Basis for calculations. Each band was presumed to reflect a specific telomere array, and the bands in the ensuing text are referred to, interchangeably, as telomere array bands or telomere arrays. Telomere arrays resolved by Southern blotting consist of the complementary 5′-TTAGGG-3′ sequence repeated in a tandem array plus any adjacent DNA (up to the restriction enzyme site) and are also known as telomere terminal restriction fragments.

Array band sharing. Telomere array patterns were assessed by array band sharing (AS) values to evaluate similarity among parents and progeny. The following calculation was used: $AS = 2N_{ab}/(N_a + N_b)$ where N_{ab} is the number of array bands shared between individuals a and b, N_a is the number of arrays in individual a, and N_b is the number of arrays in individual b (Dunnington *et al*. 1993). Values are reported as the average AS ± the standard error of the mean $(SEM = (AS \times (1 - AS)/N)^{0.5}$, where N was the number of arrays involved in calculating AS). Statistical significance was evaluated by comparing 95% confidence intervals (CI = 1.96 ± SEM) for overlap. Male and female progeny AS values were calculated separately for each family. Subsequent analyses combined male and female progeny within a family for a separate contrast with each parent.

Array frequency. Telomere array frequency (Af) values indicate the number of times an array band was present in the parents and progeny. Array frequency (Af = A/N) was calculated where A is the number of times a particular telomere array appears in a family and N is the number of individuals in that family. The SEM and 95% CI values were calculated as described above, and values were reported as the average Af ± SEM.

Chi-square goodness-of-fit. Chi-square goodness-of-fit values were calculated, where $\chi^2 = \Sigma (E - O)^2/E)$ (Rosner 1995). The χ^2 values were used to determine statistically significant differences from Mendelian segregation expectations ($\alpha = 0.05$). The observed (O) values are the array counts for specific telomere arrays in progeny. Expected values (E) were computed using several different assumptions of parental telomere array genotypes, specifically assuming parents were homozygous (as would be expected from an inbred line) or heterozygous for a particular array.

W-chromosome analysis

Chromosome preparation. Female-derived chicken embryo fibroblast cultures (Swanberg & Delany 2003) were treated with 0.07 μg Colcemid (Gibco) per ml of media for 1 h. Following trypsinization, cell suspensions were treated with hypotonic solution (0.56% KCl) for 30 min and fixed several times using 3:1 methanol:glacial acetic acid. Cells were applied to glass slides by standard techniques and stored at −80°C.

Bacterial artificial chromosome (BAC) clone. A W-specific BAC containing the SPINW gene (http://poultry.mph.msu.edu/resources/Resources.htm#bacdata, TAM32 25I6) from a UCD 001 EcoRI library (Lee *et al*. 2003) was obtained (Texas A & M University Genefinder Genomic Resources). The BAC was plated on 7.5% Luría Broth (LB) agar (10 g tryptone, 5 g yeast extract, 10 g sodium chloride, up to 1 L water, pH to 7.0 with 5 N NaOH, 1.5% Bacto Agar) containing 12.5 μg/ml chloramphenicol. Single colonies were cultured in 5 ml LB (as described but with no agar) with 12.5 μg/ml chloramphenicol (LB/chlor), and then inoculated in 100 ml LB/chlor batch cultures. The BAC DNA was extracted with the Qiagen Midi-Prep Kit according to the protocol for very low-copy plasmid/cosmid purification. For use as a FISH probe, the BAC DNA was digested by *Not* I and labeled with the DIG-Nick

Translation Kit (Invitrogen) and purified through Quick Spin Sephadex G-50 Columns (Roche Molecular Biochemicals).

Dual color fluorescence in situ *hybridization*. Slides were pre-treated according to standard procedures (Daniels & Delany 2003, Romanov *et al*. 2005). Each slide was hybridized with 100 ng DIG-labeled *SPINW*-BAC DNA and 20 μl PNA-telomere probe labeled with fluorescein (Applied Biosystems). A sheep anti-DIG rhodamine-labeled antibody (Applied Biosystems, 0.7 μg/slide) was used to detect the hybridized BAC DNA. Slides were counterstained with DAPI Vectashield solution (Vector Labs) and analyzed with an Olympus BX60 microscope using SimplePCI software (Compix Inc., Imaging Systems). Images were analyzed specifically for telomere signal intensity (green) on the W-chromosome (blue) relative to the telomere signals throughout the genome as well as in regard to the location of the *SPINW*-BAC signal (red).

Results

Ultra-long telomere profiles

Initial screening of the parental generation demonstrated substantial heterogeneity of Class III telomere arrays. Of the 20 individuals examined, none shared the same telomere array profile. Male and female parents, who were also siblings, were selected for mating pairs on the basis of similarity of telomere array patterns, differing by 1–2 array bands. Progeny array patterns (four families) were compared to those of their parents on the same gel (Figure 1). Duplicate gel runs were conducted to confirm reproducibility of patterns, and a subset of samples were run according to the three conditions outlined in the methods to resolve band sizes and band-calling. Only telomere arrays above 200 kb were analyzed. Array bands in a region were given alphabetical designations from the largest (A, 2.8 Mb) to smallest (K, 225 kb) (Figure 1) and bands within a region were assigned prime designations for further discrimination (K, K′, K″, K‴) evident by running the samples using the three conditions. In all, 28 different telomere array bands were resolved. The largest array (A) was 2.8 Mb and was present in all females (n = 58) and absent from all males (n = 46).

Expectations for a highly inbred line are that 99–100% of individuals share complete genomic identity including the telomeric DNA regions of the genome. However, of the entire data set of 104 individuals (eight parents and 96 progeny) only four individuals shared array patterns. These were from Family 1 and consisted of two sets of two male progeny; each set with identical patterns, but the sets were different from each other. Only three arrays (D, F, and G) were consistently inherited by all progeny in all families (Figure 1).

Inheritance patterns of ultra-long telomere arrays

Telomere array band sharing. On average 19 different arrays were scored per family (range of 17–21). Array sharing between progeny and their parents was expected to be 1.0. However, the average AS for the four families was 0.70 ± 0.09 (the female array A was excluded). No statistically significant differences were found between families. For all families the average combined progeny AS with the female parent (overall family average of 0.68 ± 0.09) was consistently lower than with the male parent (overall family average of 0.72 ± 0.09). Even on exclusion of array A, the male parents exhibited about one less array (average of 7.9 arrays, range of 5–11 arrays per male) than that of female parents (average of 8.7 arrays, range of 6–11 arrays per female), and this likely contributed to the AS differences when comparing the progeny to the male versus the female parent.

Telomere array transmission frequency. Figure 2 presents the frequency of the arrays in progeny averaged over all the bands studied within each family, and in the context of the parental patterns. The x-axis categories “both”, “female”, “male”, and “neither” represent the parental genotypes. The category “both” includes those arrays found in progeny that appear in both of the parents. The “female” and “male” categories include those arrays found in progeny that were present either in the female or the male parent (but not both). The “neither” category indicates the frequency of the arrays found in progeny that were not present in either parent, i.e., the novel arrays. No statistically significant differences in array frequency were observed between male and female progeny examined with regard to parental genotype (x-axis) as shown by overlapping 95% CI values (Figure 2).

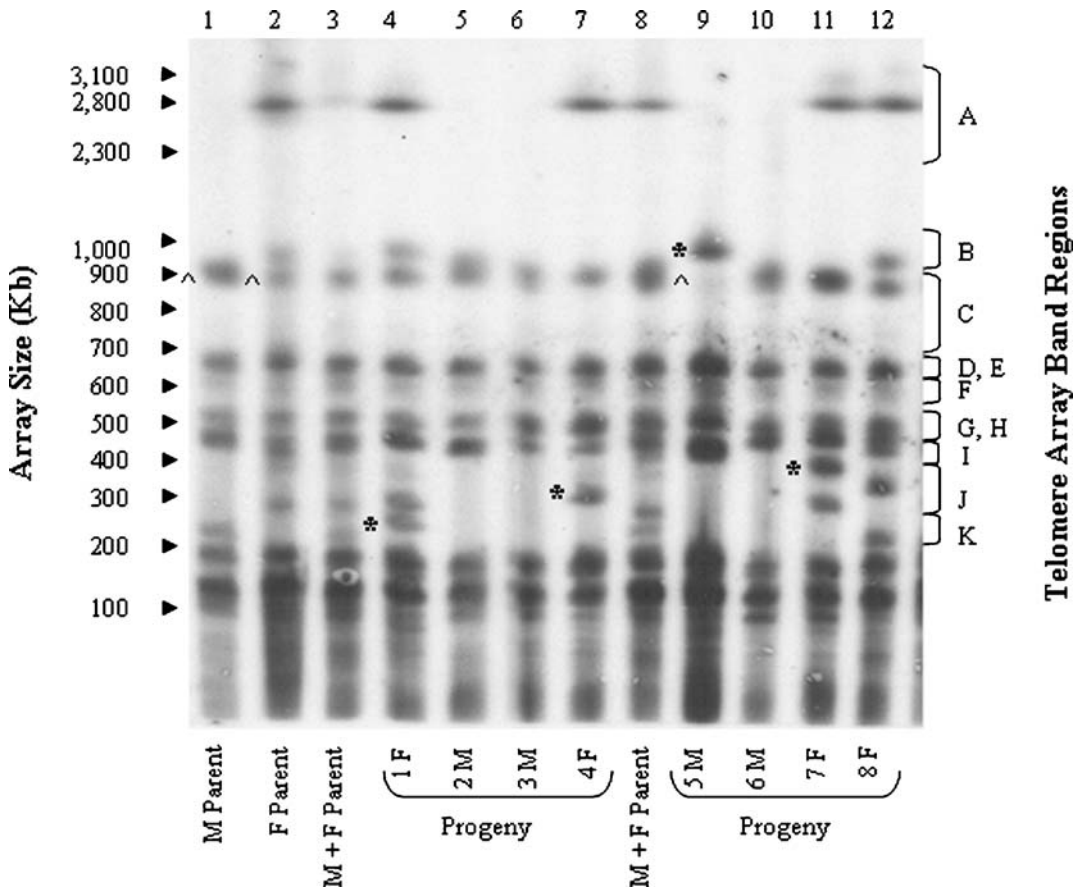
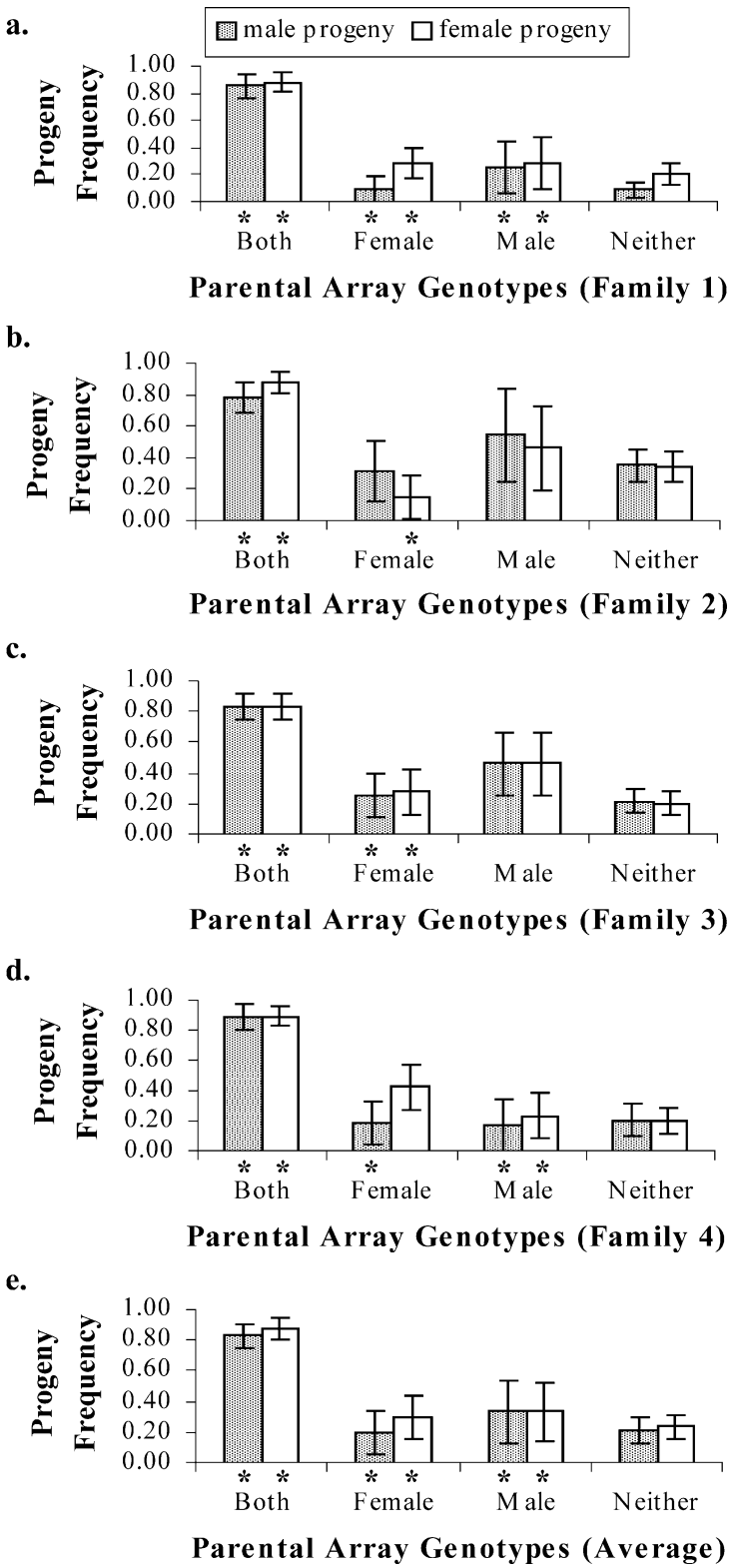


Figure 1. Ultra-long telomere arrays exhibit hypervariability unexpected for a highly inbred line. Telomere arrays and their inheritance patterns were studied in four families of an inbred line (UCD 003) by Southern blot analysis using a telomeric DNA probe. Shown here is a representative blot from one family (two parents with eight of their 24 progeny). Lanes 1 and 2 are the male (M) and female (F) parents, respectively, of progeny shown in lanes 4–7 and 9–12; lanes 3 and 8 consist of the combined parental DNA. Telomere array regions were designated A through K and 28 arrays were studied in all. Bands within a region were resolved by different gel conditions. All samples were analyzed following separation by PFGE using both Conditions 1 and 2 as described in the Methods (results from Condition 2 are shown here), and a subset of females were analyzed using Condition 3 to size the largest array (A, 2.8 Mb) which was found exclusively in females at 100% frequency. These analyses showed that parents and progeny did not exhibit identical telomere array patterns as would be expected of a highly homozygous line, and further that a number of array sets were not transmitted according to Mendelian segregation expectations. Telomere arrays D, F and G were the only arrays found in every individual of the parent/progeny data set (n = 104). In the blot shown, examples of novel arrays observed in progeny but absent from the parents are indicated by an "*" to the left of the lane; example of an array present in both parents but absent in one or more progeny is indicated by "^" to the left of the lanes.

Given the inbred status of the line (F > 0.99), all telomere arrays were expected to be inherited by 100% of the progeny (Af 1.0). However, arrays found in both parents did not meet this expectation (Figure 2). Progeny Af values ranged from 0.78 to 0.89 among the families. Since transmission did not fit a model for homozygous parents, the progeny Af of parental genotype category "both" was compared to Mendelian expectations for (1) heterozygous parents or (2) one heterozygous and one homozygous parent. In either case, arrays were not transmitted as expected. Telomere arrays found in both parents should appear in 75% of the progeny (both parents heterozygous) or in 100% of progeny (one parent heterozygous, one homozygous). The average family Af was 0.85 ± 0.03 (Figure 2, parental genotype: "Both"), which was significantly higher than expected for heterozygous parents and significantly lower than


a.
male progeny
female progeny
Progeny Frequency
1.00
0.80
0.60
0.40
0.20
0.00
Both
Female
Male
Neither
Parental Array Genotypes (Family 1)
b.
Parental Array Genotypes (Family 2)
c.
Parental Array Genotypes (Family 3)
d.
Parental Array Genotypes (Family 4)
e.
Parental Array Genotypes (Average)

the expected if only one parent was heterozygous. However, on exclusion of arrays D, F, and G from the analysis (which showed no variation), the average Af was 0.69 ± 0.03, which was not statistically different from an Af of 0.75, the expectation if both parents were heterozygous.

The progeny Af of parental genotype categories "male" and "female" (arrays found only in one parent) were compared to Mendelian expectation for one heterozygous parent exhibiting the array and one alternate homozygous parent (array missing), Af of 0.5. The Af values ranged from 0.10 to 0.40 when only the female parent exhibited the telomere array, and values were significantly different in most cases from the expected 0.50 (Figure 2, parental genotype: female). Statistical significance varied by family when only the male parent exhibited the array (Figure 2, parental genotype: male), with significant differences from expectation in family 1 and family 4 (Af = 0.20). Note that if one parent was homozygous (both homologs possess the array) and the other parent was an alternate homozygote (missing the array), the expectation for progeny would be 1.0 (100% should exhibit the array), which was clearly not observed.

The Af was determined for the category of arrays wherein neither parent exhibited the array(s) (parental genotype "neither"). Such new telomere arrays are shown in Figure 1 (lanes 4, 7, 9, and 11, denoted by "*"). Expectation for this category is zero; yet novel arrays were found in frequencies of 0.09–0.35 among families (Figure 2a–d), with an overall family average of 0.23 ± 0.03 (Figure 2e). Novel telomere arrays appeared in males as often as females in three of four families. Family 1 was the exception as novel arrays appeared in females two times more often than in males (note that family 1 had the two sets of males exhibiting identical patterns).

Female-specific ultra-long telomere array and the W-chromosome

The telomere status of the W-chromosome was studied to investigate the correlation between the 2.8 Mb telomere array observed exclusively in females (Figure 1) and chromosomal telomere signals visible by FISH. Consistently, the most intense fluorescent telomeric FISH signal was found on a DAPI-bright, small, metacentric chromosome in female cells (Figure 3a,b), which was lacking in male cytogenetic preparations (data not shown). An interstitial telomeric DNA signal of more typical intensity was observed adjacent to the larger intense signal near the centromere, with a third signal at the terminus of the opposite arm (Figure 3a,c,d). The *SPINW*-BAC probe hybridized specifically to one chromosome, the chromosome exhibiting the "mega" telomere signal, confirming W-chromosome identity (Figure 3d,e).

Discussion

Here we describe the transmission behavior of the Class III telomere arrays of the domestic chicken. Analysis of the inheritance patterns in four families from a highly inbred line indicated a lack of homozygosity for the majority of the telomere arrays larger than 200 kb. Further, array transmission did not fit simple Mendelian transmission models with novel arrays generated at high frequency. The results suggest ultra-long telomere array variation is enhanced in the germline.

Genetic variation is created through mutation and recombination. A mutation-based mechanism impacting telomere length array size, i.e., the mutation of restriction enzyme sites, seems unlikely for the results shown here, given low vertebrate mutation rates of

Figure 2. Inheritance of ultra-long telomere arrays in progeny from a highly inbred line (UCD 003) presented in the context of parental genotypes shows lack of homozygosity and generation of novel arrays. Cumulative frequency (*y*-axis) of arrays observed in male and female progeny are indicated and sorted by parental array categories (*x*-axis). "Both" indicates that arrays were observed in both parents, "female" or "male" indicates that arrays were found in that parent only, and "neither" indicates the arrays were found in the progeny but not present in either parent (generation of novel arrays). Panels **a** through **d** show single family results and panel **e** shows overall averaged values. Bars indicate 95% CI, and their overlap shows there were no differences between male and female progeny. The progeny frequency for the "both" category of parental genotype was first tested against an expectation of 100% transmission (Af 1.0) since at least one (if not both) of the parents would be expected to be homozygous in a line with a 0.99 inbreeding coefficient. The categories "female" or "male" parent only (indicating arrays found only in one parent) were tested against an expectation of 50% transmission to progeny (Af = 0.50) assuming the parent with the array to be heterozygous. An "*" indicates values were statistically different from expected (p-value ≤ 0.05). See Results for further explanation of tests.

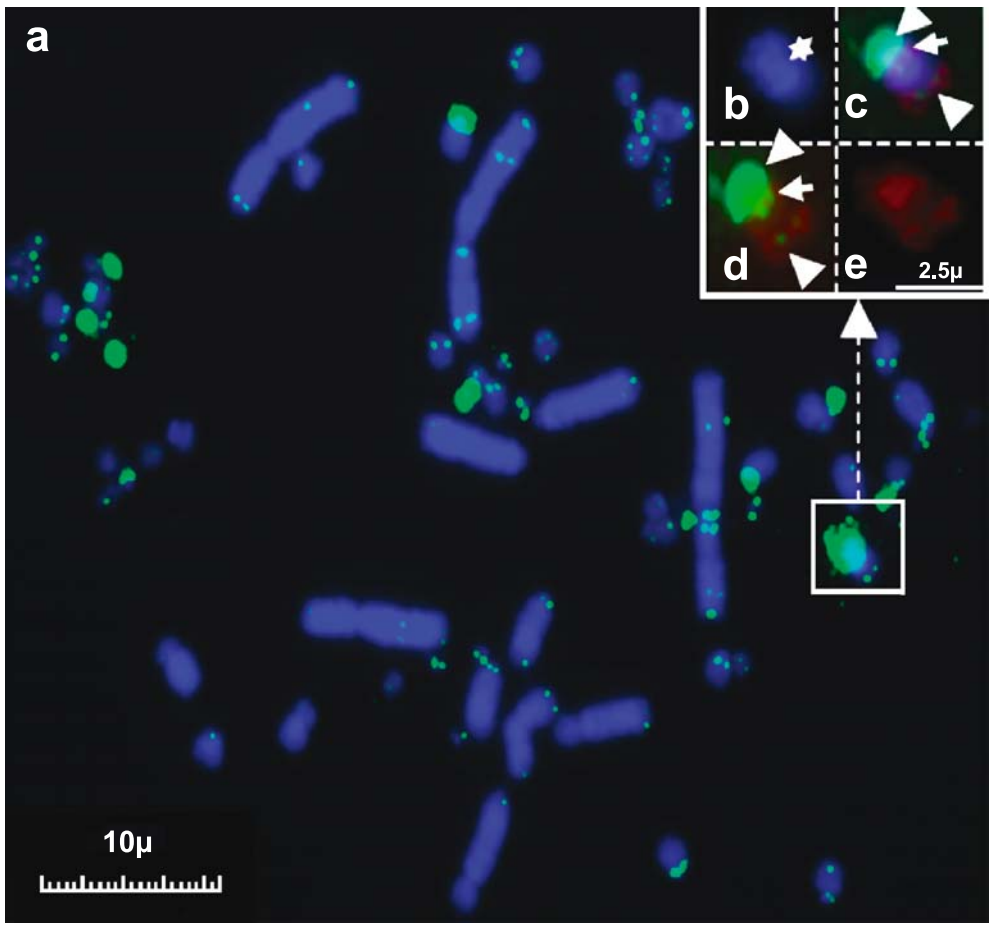


Figure 3. The female W-chromosome exhibits the largest ("mega") telomeric DNA signal in the chicken genome and its presence correlates to the ultra-long telomere (2.8 Mb) found exclusively and in all females. (**a**) DAPI-stained metaphase chromosomes (blue) are shown following hybridization with a telomere-PNA-fluorescein probe (green) indicating the extreme telomere content of the W-chromosome in comparison to other telomeric DNA signals of the genome. Panels **b**–**d** show the same W-chromosome which is boxed in panel **a**, using different fluorescence filter sets and overlays by image analysis. Each panel has the W in the same orientation. (**b**) DAPI stained W-chromosome with the centromere location indicated by a star. (**c**) Telomeric (green) and chromosome (blue) signals with the SPINW (red) fluorescence signal showing at the opposite arm, although red-only images (see panels **d** and **e**) indicate a signal along the entire chromosome. Three pairs of telomeric signals are indicated, one mega-telomere signal at the W-terminus and along the arm (arrowhead), a second telomeric signal is adjacent (interstitial) on the arm (arrow) to the mega-telomere signal, and a third signal at the opposite arm terminus (arrowhead). (**d**) The painted signal of the *SPINW*-BAC (red) is shown along with the three telomeric sites described above. (**e**) The *SPINW*-BAC (red) signal alone. The painted signal likely reflects a contribution from a repetitive element cloned in the BAC along with the *SPINW* sequences (note the W-chromosome has a very high repeat sequence content, see Schmid *et al*. 2000 for review). Others have shown *SPINW* localizes to the p terminus of the W in the pseudoautosomal region (Itoh *et al*. 2001).

10^{-3} to 10^{-6} per locus per generation (Kuhnlein et al. 1997, Broman *et al*. 1998). Telomeric sequences, both interstitial and terminal, are reported to be hot spots of recombination in both somatic and germ lineages i.e., mitotic and meiotic (Cooke *et al*. 1985, Allshire *et al*. 1989, Kipling & Cooke 1990, Starling *et al*. 1990, Kipling *et al*. 1996, Delany *et al*. 2003 and references therein). The highly repetitive nature of the TTAGGG sequence within an array could result in misalignment of telomere repeats during pairing, i.e., out-of-register-pairing (ORP); and, if accompanied by recombination, then copy number changes would be the consequence. There exist two telomere-specific mechanisms observed in vertebrates

with the potential to impact array size heterogeneity, and one is *de-novo* sequence addition in the germline (Kozik *et al*. 1998, Lanza *et al*. 2000). The chicken germline is telomerase positive but to date there is evidence only for length maintenance (not enlargement) of Class II telomere arrays (Taylor & Delany 2000). A second telomere-specific mechanism is the alternative lengthening of telomeres (ALT), a recombination-based pathway (Dunham *et al*. 2000); however, this mechanism, at least thus far, appears limited to telomerase-negative cancer cells. In the murine system, hypervariability and non-Mendelian inheritance were reported for large discrete-sized terminal arrays wherein inbred siblings were not identical, parents and progeny showed different patterns, and novel arrays were generated; such heterogeneity was also attributed to an ORP-recombination mechanism (Kipling & Cooke 1990, Starling *et al*. 1990, Ziljmans *et al*. 1997).

In chicken, only three sets of ultra-long arrays and the female-specific array behaved as expected. Interestingly, the position of the W-chromosome mega-telomere (see below) is likely on the chromosome arm that has no recombination potential, and may be invariant as a result. The invariant D, F, and G arrays suggest lack of susceptibility of these arrays to those mechanisms operating on the other ultra-long arrays. There are only a very few studies focusing on the regulation of vertebrate telomere length and therefore the mechanisms underlying length regulation are largely unknown. In the murine system a *trans*-acting dominant factor was observed in F_1 crosses of wild $\times$ laboratory mice, and a segregation study indicated a control locus on MMU 2 (Zhu *et al*. 1998). The substantial inter-chromosomal diversity in array size suggests that a chromosome-specific mechanism of telomere length regulation could be operating in chicken.

Several studies now provide substantial evidence that the ultra-long telomere arrays of the chicken are located on microchromosomes (Nanda & Schmid 1994, Delany *et al*. 2000, Nanda *et al*. 2002, Delany *et al*. 2003, this report Figure 3, and Delany *et al*. 2005). The microchromosome set of the chicken genome exhibits higher recombination rates (cM/Mb) than the macrochromosome set (3–5-fold higher) (ICGSC 2004). It is interesting to speculate that the ultra-long telomere arrays may be a contributing factor to higher recombination rates and ultimately to the generation of genetic variation. Another function of biological significance for the ultra-long arrays could be a protective buffer against telomere attrition and erosion (Delany *et al*. 2000, Taylor & Delany 2000, Swanberg & Delany 2003) of nearby genes as the microchromosomes are gene-dense compared to the macrochromosomes (Smith *et al*. 2000, ICGSC 2004).

A novel finding of this study was the association of the largest telomere array (2.8 Mb, female-specific) with a "mega" telomeric signal on the W-chromosome, as compared to the other telomeric signals in the genome (see Figure 3a). The *SPINW* gene contained within the BAC used to identify the W-chromosome, maps to the pseudoautosomal region (PAR) on the p arm of the W (Itoh *et al*. 2001). The *SPINW*-BAC produced a painted signal over the chromosome likely as a result of repetitive elements adjacent to *SPINW* cloned into the BAC. The positioning of the PAR on Wp and the current view of the W cytogenetic map (Schmid *et al*. 2005) are based on heterochromatin location by C-banding, FISH mapping of EcoRI, Xho I, SspI repeat families and single gene loci (e.g., Wpcki), and meiotic pairing analysis of the Zp and Wp (Suka *et al*. 1993, Itoh *et al*. 2001, Itoh & Mizuno 2002, Nanda *et al*. 2002). We predict that the mega-telomeric signal of the W is the q arm telomere (non-PAR arm) terminal to the EcoRI repeat family (see Schmid *et al*. 2005, Figure 30).

The chicken genome sequence assembly indicates a deficit of W-chromosome sequence with only ~217 kb reliably assigned to the W (ICGSC 2004). This is due to its enormous repeat content (Schmid *et al*. 2000, 2005) and the difficulties in cloning, sequencing, and assembling such elements (ICGSC 2004). The W should have at least ~23 Mb of sequence given that it is the ninth largest chromosome and thus similar to GGA 9 which has that amount of assembled sequence. Itoh & Mizuno (2002) suggest that the W could be as large as 54 Mb. Here we add at least another 3 Mb of telomeric DNA.

In summary, this study provides experimental evidence that the majority of ultra-long telomere arrays of the chicken genome exhibit hypervariability as a result of meiotic instability. The mechanism we favor is that invoked for the mouse large telomere arrays (and other tandem repeat families, see Delany *et al*. 1991, Miller *et al*. 1996); that is, ORP coupled with enhanced meiotic recombination, generating arrays of new copy number, although, given the current state of knowledge, other telomere-based mechanisms cannot be ruled out as contributing factors.

We also report that the largest telomere array maps to the W-chromosome. These characteristics expand the nature of telomere array function in the chicken genome beyond that of the telomere clock. Chicken telomere arrays may be involved in sex chromosome organization and the generation of genetic variation at chromosome termini, the functional significance of which requires analysis in a non-inbred line and knowledge of adjacent loci.

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References

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

Allshire RC, Dempster M, Hastie ND (1989) Human telomeres contain at least three types of G-rich repeats distributed non-randomly. *Nucleic Acids Res* **17**: 4611–4627.

Blackburn EH (1994) Telomeres: no end in sight. *Cell* **77**: 621–623.

Broman KW, Murray JC, Sheffield VC, White RL, Weber JL (1998) Comprehensive human genetic maps: Individual and sex-specific variation in recombination. *Am J Hum Genet* **63**: 861–869.

Cooke HJ, Brown WRA, Rappold GA (1985) Hypervariable telomere sequences from the human sex chromosomes are pseudoautosomal. *Nature* **317**: 687–692.

Crittenden LB, Provencher L, Santangelo L *et al*. (1993) Characterization of a red jungle fowl by white leghorn backcross reference population for molecular mapping of the chicken genome. *Poultry Sci* **72**: 334–348.

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

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

Delany ME, Gessaro TM, Rodrigue KL, Daniels LM (2005) Cytogenomic approach to mapping the mega-telomere arrays of the chicken genome. In *Workshop on Chicken Genomics and Development*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press (abstract).

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, Muscarella DE, Bloom SE (1991) Formation of nucleolar polymorphisms in trisomic chickens and subsequent microevolution of rRNA gene clusters in diploids. *J Heredity* **82**: 213–220.

Dhaene K, Van Marck E, Parwaresch R (2000) Telomeres, telomerase and cancer: an up-date. *Virchows Arch* **437**: 1–16.

Dunham MA, Neumann AA, Fasching CL, Reddel RR (2000) Telomere maintenance by recombination in human cells. *Nat Genet* **26**: 447–450.

Dunnington EA, Stallard LC, Siegel PB, Hillel J (1993) Evaluating linkage between DNA fingerprint bands and quantitative traits in chickens: interactions. *Poult Sci* **72**: 1000–1004.

Forsyth RN, Wright WE, Shay JW (2002) Telomerase and differentiation in multicellular organisms: turn it off, turn it on, and turn it off again. *Differentiation* **69**: 188–197.

Greider CW, Blackburn EH (1989) A telomeric sequence in the RNA of *Tetrahymena* telomerase required for telomere repeat synthesis. *Nature* **337**: 331–337.

Harley CB, Futcher AB, Greider CW (1990) Telomeres shorten during ageing of human fibroblasts. *Nature* **345**: 458–460.

Henderson S, Allsopp R, Spector D, Wang SS, Harley C (1996) *In situ* analysis of changes in telomere size during replicative aging and cell transformation. *J Cell Biol* **134**: 1–12.

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

Itoh Y, Mizuno S (2002) Molecular and cytological characterization of SspI-family repetitive sequence on the chicken W chromosome. *Chromosome Res* **10**: 499–511.

Itoh Y, Hori T, Saitoh, H, Mizuno S (2001) Chicken spindlin genes on W and Z chromosomes: transcriptional expression of both genes and dynamic behavior of spindling in interphase and mitotic cells. *Chromosome Res* **9**: 283–299.

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

Kipling D, Wilson HE, Thomson EJ *et al*. (1996) Structural variation of the pseudoautosomal region between and within inbred mouse strains. *Proc Natl Acad Sci USA* **93**: 171–175.

Kozik A, Bradbury E, Zalensky A (1998) Increased telomere size in sperm cells of mammals with long terminal (TTAGGG)n arrays. *Mol Reprod Dev* **51**: 98–104.

Kuhnlein U, Zadworny D, Dawe Y, Fairfull RW, Gavora JS (1997) Assessment of inbreeding by DNA fingerprinting: development of a calibration curve using defined strains of chickens. *Genetics* **125**: 161–165.

Lanza RP, Cibelli JB, Blackwell C *et al*. (2000) Extenstion of cell life-span and telomere length in animals cloned from senescent somatic cells. *Science* **288**: 665–669.

Lee MK, Ren CW, Yan B et al. (2003) Construction and characterization of three BAC libraries for analysis of the chicken genome. *Anim Genet* **34**: 151–152.

Miller MM, Goto RM, Taylor RL *et al*. (1996) Assignment of Rfp-Y to the chicken major histocompatibility complex/NOR microchromosome and evidence for high frequency recombination associated with the nucleolar organizer region. *Proc Natl Acad Sci USA* **93**: 3958–3962.

Mittwoch U (1998) Phenotypic manifestations during the development of the dominant and default gonads in mammals and birds. *J Exp Zool* **281**: 466–471.

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.

Romanov M, Daniels LM, Dodgson JB, Delany ME (2005) Integration of the cytogenetic and physical maps of chicken chromosome 17. *Chromosome Res* **13**: 215–222.

Rosner B (1995) Hypothesis testing: categorical data. In Kugushev A, Burger J, Purrington L, eds. *Fundamentals of Biostatistics*, 4th edn. Belmont, CA: Duxbury Press, pp. 141–190, 345–440.

Sambrook J, Russell DW (2001) Preparation and analysis of eukaryotic and genomic DNA. In: Argentine J *et al*. eds. *Molecular Cloning: A Laboratory Manual*, Vol. 1, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp 6.39–6.46.

Schmid M, Nanda I, Guttenbach M *et al*. (2000) First report on chicken genes and chromosomes 2000. *Cytogenet Cell Genet* **90**: 169–218.

Schmid M, Nanda I, Hoehn H *et al*. (2005) Second report on chicken genes and chromosomes 2005. *Cytogenet Genome Res* **109**: 415–479.

Shore D (1995) Aging. Silence is golden. *Curr Biol* **5**: 822–825.

Smith J, Bruley CK, Paton IR *et al*. (2000) Differences in gene density on chicken macrochromosomes and microchromosomes. *Anim Genet* **31**: 96–103.

Starling JA, Maule J, Hastie ND, Allshire RC (1990) Extensive telomere repeat arrays in mouse are hypervariable. *Nucleic Acids Res* **18**: 6881–6888.

Stavenhagen JB, Zakian VA (1998) Yeast telomeres exert a position effect on recombination between internal tracts of yeast telomeric DNA. *Genes Dev* **12**: 3044–3058.

Suka N, Shinohara Y, Saitoh Y *et al*. (1993) W-heterochromatin of chicken; its unusual DNA contents, late replication, and chromatin structure. *Genetica* **88**: 93–105.

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

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

Zakian VA (1995) Telomeres: beginning to understand the end. *Science* **270**: 1601–1607.

Zhu L, Hathcock KS, Hande P, Lansdorp PM, Seldin MF, Hodes RJ (1998) Telomere length regulation in mice is linked to a novel chromosome locus. *Proc Natl Acad Sci USA* **95**: 3648–3659.

Ziljmans JMJM, Martens UM, Poon SSS *et al*. (1997) Telomeres in the mouse have large inter-chromosomal variations in the number of T_2AG_3 repeats. *Proc Natl Acad Sci USA* **94**: 7423–7428.