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Abrupt shortening of bird W chromosomes in ancestral Neognathae

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As a result of suppressed recombination, heterogametic sex chromosomes (either Y or W) are usually assumed to gradually shorten over evolutionary time as a way to remove accumulated mutations. However, suppressed recombination removes the most obvious mechanism for excising portions of sex chromosomes. We examined ratios of W/Z chromosome size across 224 bird species from 146 genera. Much of the data were obtained from a previous study (Rutkowska *et al.* 2012. *Biology Letters* **8**: 636–638), who, similar to ourselves, found no gradual decrease in W chromosome length over evolutionary time. However, we show an abrupt decrease in W chromosome length at or just after the phylogenetic split between the two extant bird superorders, Paleognathae and Neognathae, indicating that the key to understanding sex chromosome evolution may have little to do with gradual suppression of recombination. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **119**, 488–496.

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INTRODUCTION

It is almost universally assumed that heteromorphic sex chromosomes (i.e. where the Y or W chromosome is notably shorter than the X or Z chromosome) evolved from autosomes (Muller, 1932; Rice, 1987; Charlesworth & Charlesworth, 2000; Bachtrog, 2008; Bergero & Charlesworth, 2009). The evolution of heteromorphic sex chromosomes is considered to have started with differentiation between two homologues at one or a few loci followed by the gradual evolution of shorter Y or W chromosomes (Charlesworth, 1991; Solari, 1994; Gorelick, 2003; Uller & Badyaev, 2009).

The hypothesis suggesting that the sex chromosomes of birds and mammals originated from a common ancestral chromosome in reptiles (Matsubara *et al.*, 2006) has largely been substantiated. Molecular genetics has shown probable universality in the mechanism of avian sex chromosome evolution from a shared autosomal origin to the highly heteromorphic homologues observed in modern passerine birds (Ogawa, Murata & Mizuno, 1998; Mank & Ellegren, 2007; Tsuda *et al.*, 2007). Basal birds in the superorder Paleognathea have a proto-Z chromosome that was inherited from a reptilian ancestor and was retained in both ratite and tinamou lineages (Smith & Voss, 2007).

Bird and mammal sex chromosomes are also hypothesized to have experienced subsequent gradual diminution in Y or W chromosome size following their split from their common ancestor (Smith & Voss, 2007). Z chromosome length is almost constant across all extant birds, suggesting that bird sex chromosomes evolved from autosomes through the gradual shortening of the W chromosome, and not by lengthening of the Z chromosome (Graves & Shetty, 2001). The question remains, however, whether there is a pattern to the decrease in W chromosome size, and, if a pattern exists, whether this is gradual or abrupt. All evolutionary models, such as Muller's ratchet (Muller, 1964; Nei, 1970; Gillespie, 2004), Kondrashov's hatchet (Gillespie, 2004), genetic hitchhiking (Rice, 1987), and retrotransposon traps (Steinemann & Steinemann, 1992), model the shortening of Y or W

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chromosomes as an evolutionary process with gradual functional degradation as a result of the accumulation of deleterious mutations. Most assume that this will directly result in gradual structural degradation of chromosomes because there is a selective advantage to no longer replicating loci that are not functional (Charlesworth, 1978; Jablonka & Lamb, 1990) and nucleic acids are expensive to produce, and hence there is an ecological nitrogen and phosphorus limitation. However, Rutkowska, Lagisz & Nakagawa (2012) showed that shortening of W chromosomes in birds was not a gradual process. Furthermore, Zhou *et al.* (2014) found that the loss of recombination on avian W chromosomes was an episodic and sometimes abrupt process.

Although gradual diminution of W chromosome length is almost always predicated on gradual reduced recombination and consequent gradual accumulation of mutations, there is no obvious mechanism for excising such mutations and no strong link between a lack of recombination and the shortening of sex chromosomes (Zhou *et al.*, 2014). Although much is known about the mechanisms that suppress recombination, such as chromosomal inversions (Kirkpatrick, 2010; McGaugh & Noor, 2012), the mechanism(s) by which suppressed recombination results in shorter sex chromosomes are far less clear.

There is almost no rationale in the published literature suggesting that avian sex chromosome evolution might be abrupt. However, a spate of recent work indicates that the evolution of avian sex chromosomes should differ between the superorders Paleognathae and Neognathae. First, a recent molecular cytogenetic study showed that the (paleognathic) ratites and tinamous have maintained an 'ancestral' karyotype (Nishida-Umehara *et al.*, 2007). Second, for birds, only the Neognathae lack large areas of meiotic recombination between their Z and W chromosomes, except in a short pseudo-autosomal region, whereas ratites largely maintain recombination along most of their W chromosomes (Pigozzi & Solari, 2005; Nishida-Umehara *et al.*, 2007). Tinamous lack recombination along some portions of their W chromosomes, although this pattern is distinct in being much less localized and less extensive than in Neognathae (Pigozzi & Solari, 2005; Pigozzi, 2011). Third, the cessation of recombination has been inferred in the Neognathae, for several discrete periods of approximately 15 Myr each, with no such cessation of recombination in the Paleognathae (Nam & Ellegren, 2008). Fourth, in other metazoa, W and Y chromosomes almost never gradually diminish in length to non-existence; rather, XX/X0 and ZZ/Z0 usually arise via abrupt losses of a large portion of the sex chromosome (Hughes *et al.*, 2012). These recent publications hint that the evolution of sex chromosomes may have been abrupt at the superorder split in birds.

Currently, insufficient data exist on nuclear substitution rates of avian W chromosomes; hence, *sensu* Rutkowska *et al.* (2012), we used overall substitution rates across the nuclear genome for phylogenetic and nonphylogenetic analyses. Moreover, it would be difficult to estimate substitution rates in the face of gene excisions that ostensibly make W and Y chromosome shorter than Z and X over evolutionary time.

Rutkowska *et al.* (2012) did not test for a step change in W chromosome length at or just after the paleogath/neognath split. Therefore, we used their data, as well as data that we collected from further published karyotype data using the same karyotype image analysis methods employed by Rutkowska *et al.* (2012), to look for an abrupt diminution in W chromosome size.

MATERIAL AND METHODS

The ratio of W/Z chromosome length was either obtained from Rutkowska *et al.* (2012) for 200 bird species or using their methodology for 55 bird species (29 of which they reported and 26 that they had not reported). Both Rutkowska *et al.* (2012) and ourselves measured the length of W and Z chromosomes from published karyotypes in which both W and Z chromosomes appeared within the same image.

We used two alternative approaches in an attempt to calibrate the above method. First, we measured not only the length of published karyotype images, but also area and optical density (hereafter 'density') of the same karyotype images (see Supporting information, Table S1). Second, we had hoped ratios of W/Z chromosome length from karyotypes could be compared with the ratio of the number of nucleotides sequenced on W and Z. Although there are purported to be full sequences for several bird species in the National Centre for Biotechnology Information's (NCBI) Genome Information by Organism database (http://www.ncbi.nlm.nih.gov/genome/browse/), sequencing is still far from complete for sex chromosomes for any bird species; hence, we relied on W/Z size ratios from published karyotypes.

Chromosome length was determined using an automatic ruler in PAINTSHOP PRO X2 (Corel). Although it is technically simple to measure lengths of W and Z karyotypes, we were concerned that projection, folding, and squashing of chromosomes might make length an unsatisfactory proxy. We therefore measured area and density because these size measures account for idiosyncratic projections of the

two-dimensional karyotypic images that are obtained from chromosomal length measurements. To measure area, we converted all components of the image exceeding a set brightness tolerance to black (pixel value 256) and removed all other components. To measure density, we used MATLAB (MathWorks Inc.) to convert each image into a numerical value representing the sum of the pixel values for each image. We computed mean W/Z ratios for each species.

We computed cumulative branch length (accumulated mutations) as the total number of nucleotide substitutions that have occurred since the root of the avian phylogenetic tree (Hackett *et al.*, 2008). We took number of nucleotide substitutions since the root directly from the data file kindly provided (Rebecca Kimball, personal communication). For the unrooted tree, we verified numerical values of these substitution rates by measuring branch lengths from figure S1 in Hackett *et al.* (2008) but then used their rooted tree for all subsequent analyses. Although there may be errors in estimates of the cumulative number of nucleotide substitutions per site, such as might occur by having the wrong topology of the phylogenetic tree, we nonetheless considered it to represent an independent variable.

Nonphylogenetic comparative methods

We used generalized linear models (GLMs) to test for a difference in W/Z chromosome size between palaeognaths and neognaths using R, version 3.2.1 (R Foundation for Statistical Computing). We ran all models at the species taxonomic level. We opted for the Benjamini–Hochberg-Yekutieli false discovery rate adjusted α instead of the Bonferroni adjustment because the Bonferroni adjustment is overly conservative (Benjamini & Hochberg, 1995; Benjamini & Yekutieli, 2001; Nakagawa, 2004; Narum, 2006). Because there is no obvious way to parse statistical error between errors in the number of substitutions per site and evolutionary stochasticity in the W/Z chromosome size ratio, we used model I (vice model II) regressions (Sokal & Rohlf, 1995; Grafen & Hails, 2002). Our analysis differs from the previous study of W/Z chromosome size by Rutkowska *et al.* (2012) in that we included superorder as an independent variable in our models, thus directly testing for both abrupt and gradual changes in chromosome size. We did not use phylogenetically controlled regressions to test for difference in W/Z chromosome size between the two bird superorders because our question is a phylogenetic one, and removing phylogenetic signal would erase any potential signal for change between the superorders.

Phylogenetic comparative methods

We tested for phylogenetic signal in the residuals from regressions of our various chromosome size metrics against the accumulated number of nucleotide substitutions per site using Pagel's λ (Pagel, 1999; Freckleton, Harvey & Pagel, 2002) in the phytools R package (Revell, 2011). We used residuals in our tests for a phylogenetic signal because, similar to ordinary least squares regression that assumes normality of residuals, phylogenetic comparative methods assume phylogenetic signal in the residuals (Revell, 2010). We performed all phylogenetically controlled analyses using pseudo-posterior distributions of 1000 phylogenetic tree topologies each for the four sets of analyses used in Jetz *et al.* (2012). The tree distributions were drawn from phylogenetic analysis of the avian phylogeny where the backbone was constrained according to Hackett *et al.* (2008) and Ericson *et al.* (2006). For additional information on phylogenetic tree construction, see Jetz *et al.* (2012). Given a Brownian motion process of trait evolution with a covariance matrix:

$$V\{X_i\} = \sigma^2 T_{ij} \text{ (Brownian motion model)},$$

where X_i is the variance of the base-to-tip branch length for species i, $V\{X_i\}$ is the resulting covariance matrix, T is the distance between the root of the tree and the most recent common ancestor (therefore T_{ij} is the distance between taxa i and j), and σ^2 is the variance. Pagel's λ, which varies between zero and the upper limit determined by the height of the most recent internal node, is a multiplier of the off-diagonal elements of V (Blomberg, Garland & Ives, 2003). Values of $\lambda = 0$ are indicative of phylogenetic independence, whereas high values of λ indicate Brownian evolution of a trait across a given phylogenetic tree or indicate significant phylogenetic signal (Pagel, 1999). We used a likelihood ratio test to determine the statistical significance of Pagel's λ for each set of residuals (Revell, 2010).

We used phylogenetic generalized least squares (PGLS) regression to test for significant correlations between our chromosome metrics and accumulated genome-wide mutations. All PGLS analyses were run across a pseudo-posterior distribution of 1000 tree topologies for each of the four sets of phylogenetic analyses from Jetz *et al.* (2012). Using Akaike information criterion, we selected among three evolutionary models: Brownian motion (stochasticity), Ornstein–Uhlenbeck (stabilizing selection) (Martins & Hansen, 1997; Butler & King, 2004), and Blomberg's model of varying evolutionary rates (Blomberg *et al.*, 2003).

$$\text{Brownian motion model: } V\{X_i\} = \sigma^2 T_a$$

$$\text{Ornstein-Uhlenbeck model: } V\{X_i\} = \frac{1 - d^{2(T_{ij}+T_i)}}{1 - d^2}\sigma_\gamma^2$$

$$\text{Blomberg model: } V\{X_i\} = \frac{1 - g^{-(T_{ij}+T_i)}}{1 - g^{-1}}\sigma_\gamma^2,$$

where X_i is the variance of the base-to-tip branch length for species i, $V\{X_i\}$ is the resulting covariance matrix, T is the distance between the root of the tree and the most recent common ancestor (therefore T_{ij} is the distance between taxa i and j), and σ^2 is the variance as a result of Brownian motion. For the Blomberg model of varying evolutionary rates, g is the rate of evolution. For the Ornstein–Uhlenbeck model, d describes the degree of stabilizing selection, where $d = 1$ is Brownian motion and $d < 1$ indicates strong stabilizing selection (Blomberg *et al.*, 2003).

To visualize chromosome evolution in *Aves*, we used continuous character mapping in the phytools R package (Revell, 2011). This method involves estimation of ancestral character states using a maximum likelihood approach and interpolation of states along each branch. We created character maps for one tree from each of the four pseudo-posterior distributions of tree topologies.

RESULTS

Neognaths have shorter W chromosomes than paleognaths, based on W/Z length (Fig. 1), area, and density ratios (Tables 1 and 2). Length, area, and density yield approximately identical results. The abrupt (step) change in W/Z chromosome length is apparent when mapped onto the phylogenetic tree (Fig. 2; where blue indicates W and Z chromosomes of almost equal length).

Is there a phylogenetic signal in avian W chromosome size and, if so, is the change in W chromosome size evolutionarily abrupt or gradual? We demonstrate that the residuals of W/Z chromosome length, area, and density showed significant phylogenetic signal using Pagel's λ (Table 1), confirming that W/Z size differs significant among palaeognaths and neognaths. The GLM of W/Z ratio against superorder also shows that relative W chromosome size depends on superorder (Table 2).

Using PGLS, we found no significant correlation of W/Z chromosome length, area, and density with accumulated mutations (Table 3). We did not explicitly include superorder as a separate variable in the PGLS analyses because the purpose of PGLS is to remove the signal of such phylogenetic effects.

The above results did not change qualitatively when we included the most obvious outlier from the analysis: *Phalcoboenus megalopterus* (additional results not shown; this species does not appear in Hackett *et al.*, 2008) for which data may have been

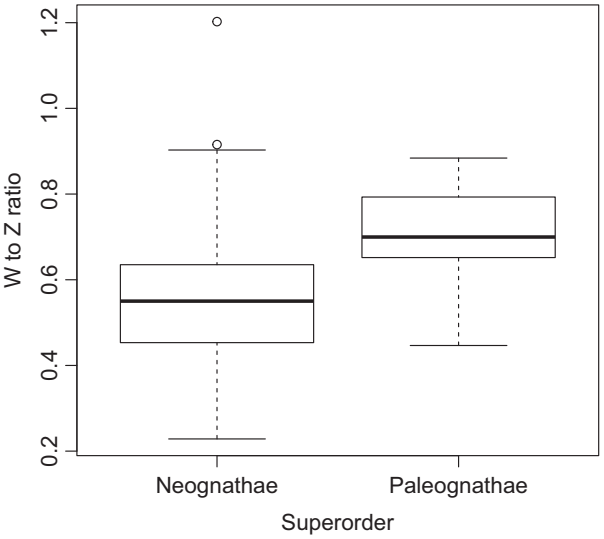

Figure 1. Boxplots of W/Z chromosome length ratios in neognaths and paleognaths. Neognaths have sex chromosomes that are significantly smaller than that of paleognaths (at the $\alpha = 0.05$ level). Boxes represent 25th and 75th percentiles, with a thick line at the 50th percentile. Whiskers show the entire range of the data excluding outliers.

Table 1. Statistically significant phylogenetic signal exists in residuals of regressions of ratio of W/Z to number of nucleotide substitutions

W/Z	Tree	Pagel's λ	*P*
Length	Ericson backbone	0.88	< 0.001
	Ericson sequenced	0.87	< 0.001
	Hackett backbone	0.88	< 0.001
	Hackett sequenced	0.87	< 0.001
Area	Ericson backbone	0.77	< 0.001
	Ericson sequenced	0.79	< 0.001
	Hackett backbone	0.77	< 0.001
	Hackett sequenced	0.78	< 0.001
Density	Ericson backbone	0.83	< 0.001
	Ericson sequenced	0.85	< 0.001
	Hackett backbone	0.88	< 0.001
	Hackett sequenced	0.87	< 0.001

The *P*-values are calculated as the fraction of the 1000 trees that are not significant (i.e. all of the 1000 iterations were significant, hence there was no need for Benjamini–Hochberg-Yekutieli false discovery rate corrections of 0.0161 for these 12 tests). The values shown are without inclusion of superorder in the model because its inclusion would amount to double-counting (Ericson *et al.*, 2006; Hackett *et al.*, 2008).

Table 2. Generalized linear model of W/Z ratio against superorder shows that W chromosome size is correlated with superorder

Dependent variable: ratio of W/Z	R^2_{adjusted}	*t*-test (*P*)	Parameter estimate
Length	0.045	**0.001**	0.166
Area	0.104	**0.009**	0.184
Density	0.083	**0.019**	0.171

Bold indicates statistical significance at $\alpha = 0.05$ with a Benjamini–Hochberg-Yekutieli false discovery rate adjusted α-level of $P < 0.03$ to account for the three tests performed. Regressions for area and density are over 55 species, in 30 families. Regressions for length are over 90 species, in 30 families.

erroneous: 'The Z [chromosome] could not be identified with certainty' (Belterman & de Boer, 1990: 25).

DISCUSSION

Given relative constancy of Z chromosome size in extant birds, data on the ratio of W/Z chromosome size in birds reflect changes in W chromosome size. Rutkowska *et al.* (2012) showed that avian W chromosomes did not become shorter in a gradual fashion per Muller's ratchet, Kondrashov's hatchet, genetic hitchhiking, and retrotransposon traps. We extended their results by showing that much of this decrease in W chromosome size occurred near the paleognath/neognath split (Figs 1, 2; Tables 1, 2). Using PGLS, we also found that W chromosome length did not diminish gradually with increasing number of accumulated nucleotide substitutions (Table 3), which is in agreement with Rutkowska *et al.* (2012), even though they did not explicitly examine nucleotide substitution rates.

Our results for chromosome length, area, and density are all approximately the same (Tables 1, 2, 3). If anything, this reflects the great care taken by cytologists in composing karyotypes, ensuring that chromosomes are nicely flattened and not foreshortened in cell squashes.

The number of nucleotide substitutions in our analysis was based on work by Hackett *et al.* (2008) on 19 loci spread across the genome, not necessarily substitution rates specifically on the Z or W chromosome, for which sufficient data do not yet exist. We therefore assumed that nucleotide substitution rates are proportional (albeit not necessarily equal) across all chromosomes, an assumption also invoked by Rutkowska *et al.* (2012). This is consistent with the findings of a study by Ross (2006) showing that, over evolutionary timescales, nucleotide substitution rates are largely determined by environmental factors, as well a study by Charlesworth, Jordan & Charlesworth's (2014) reporting that genes in pseudoautosomal regions of Z and W chromosomes evolve in a similar manner to autosomal genes. There are three problems related to only examining nucleotide substitution rates on the W chromosome, with the rationale that this is what should drive gradual diminution of sex chromosome size. First, accumulated mutations on the W chromosome are excised, thereby biasing mutation estimates for the W chromosome. It may be better to assume that, for a given taxon, mutation rates are approximately constant across the genome (Ross, 2006). Second, recombination is suppressed on parts of the W chromosome. Third, there are currently insufficient data available on nucleotide substitution rates of avian W chromosomes. Although we acknowledge these problems, the data of Hackett *et al.* (2008) concerning the mean number of nucleotide substitutions across the genome currently provide the best measure of cumulative branch length for birds. Eventually, richer data on the number of nucleotide substitutions on the W chromosome may replace the analysis in the present study, although only if those data are somehow adjusted for presumed excisions of accumulated nucleotide substitutions and a lack of recombination from the ancestral W chromosome. In the meantime, using autosomal nucleotide substitution rates to examine whether the evolution of sex chromosome size is gradual appears to be the best approach, which is likely why the approach was also adopted by Rutkowska *et al.* (2012).

Three neognath species (*Glaucidium radiatum*, *Neochmia phaeton*, and *Nyctibius griseus*) have W chromosomes that appear to be larger than their Z chromosomes (Misra & Srivastava, 1974; Christidis, 1986; Nieto *et al.*, 2012). We are not certain whether those data reflect improper assignment of which chromosome is W versus Z or whether W really has grown longer over evolutionary time, as with UV chromosomes of some mosses (McDaniel *et al.*, 2013). Longer W or Y chromosomes (longer than Z and X) are called neo-sex chromosomes and are usually considered to be the result of an autosome fusing with a W or Y chromosome (Beukeboom & Perrin, 2014). The fairly long W chromosomes in the strigiforme *Tyto alba* and the passerines *Columbina talpacoti* and *Turdus migratorius* (Fig. 2; see also Supporting information, Table S1) may possibly be a result of such fusions. Neo-sex chromosomes appear to be very rare in birds and hence do not affect the overall conclusion that there was probably an abrupt shortening of W near the paleognath/neognath split.

Work on the evolution of heteromorphic sex chromosomes has recently reflected older works on

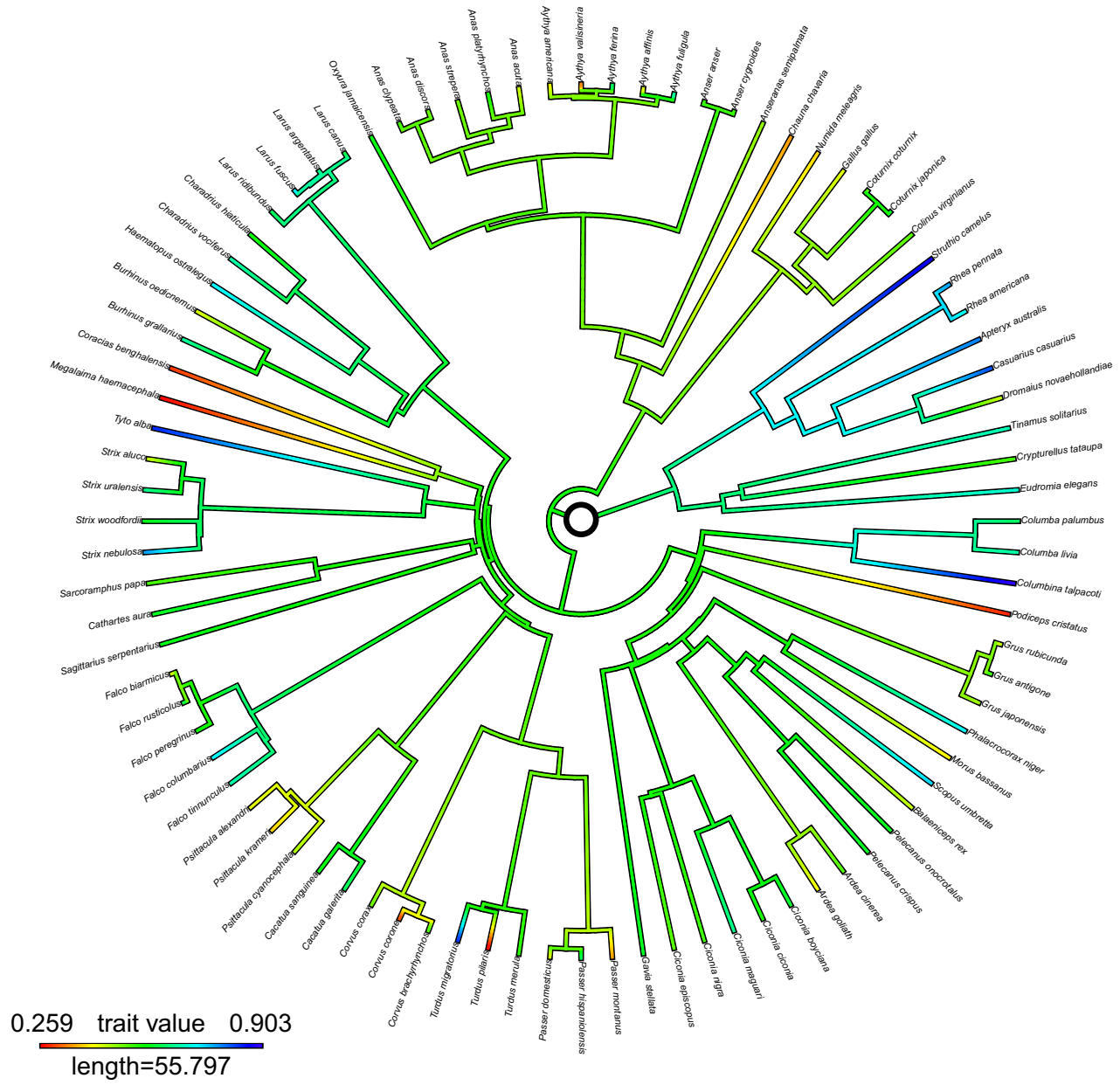

Figure 2. Neognaths have smaller W/Z chromosome length ratios than paleognaths. W/Z length ratios for 90 bird species mapped onto one tree topology from a posterior distribution of trees constrained using the backbone of the Hackett *et al.* (2008) phylogeny. The tree is taken from Jetz *et al.* (2012). The unfilled circle marks the split between palaeognaths and neognaths. Cool colours (e.g. blue) indicate near equal length W and Z chromosomes, whereas warm colours (e.g. red) indicate smaller ratios of W/Z chromosome lengths.

evolutionary rate (Wright, 1931; Eldredge & Gould, 1972), investigating whether the size reduction of Y or W chromosomes is gradual or abrupt (Charlesworth, Charlesworth & Marais, 2005; Bachtrog, 2006; Hughes *et al.*, 2012; Gamble *et al.*, 2014). Is it possible that paleognaths simply have a slower rate of W chromosome degeneration than neognaths (Yazdi & Ellegren, 2014)? What would cause such a difference in rates? Our results show an abrupt shortening of W chromosomes at or around the divergence of paleognaths and neognaths, a view that is inconsistent with theories of sex chromosome evolution via Muller's ratchet, Kondrashov's hatchet, genetic hitchhiking, and retrotransposon traps, all of which assume a gradual shortening over evolutionary time of Y and W chromosomes, but consistent

Table 3. Phylogenetic generalized least squares analysis shows there is no significant correlation of W/Z chromosome length, area, and density with accumulated mutations

Dependent variable	Tree distribution	Model	*t*	*P*	AIC	ΔAIC
Length	Ericson all species	ACDC	−0.74	1.00	−385.02	49.93
		Brownian motion	**−0.41**	**1.00**	**−434.95**	**0.00**
		Ornstein–Uhlenbeck	−3.96	0.00	−225.13	209.83
	Ericson sequenced species	ACDC	−0.70	1.00	−364.66	47.25
		Brownian motion	**−0.43**	**1.00**	**−411.90**	**0.00**
		Ornstein–Uhlenbeck	−3.96	0.00	−210.17	201.73
	Hackett all species	ACDC	−0.74	1.00	−383.42	49.79
		Brownian motion	**−0.38**	**1.00**	**−433.22**	**0.00**
		Ornstein-Uhlenbeck	−3.96	0.00	−225.08	208.13
	Hackett sequenced species	ACDC	−0.70	1.00	−363.55	46.98
		Brownian motion	**−0.40**	**1.00**	**−410.53**	**0.00**
		Ornstein–Uhlenbeck	−3.96	0.00	−210.17	200.36
Area	Ericson all species	ACDC	−1.25	1.00	−166.14	22.67
		Brownian motion	**−0.88**	**1.00**	**−188.80**	**0.00**
		Ornstein-Uhlenbeck	−3.95	0.00	−116.21	72.59
	Ericson sequenced species	ACDC	−1.29	1.00	−146.73	20.10
		Brownian motion	**−0.97**	**1.00**	**−166.83**	**0.00**
		Ornstein–Uhlenbeck	−4.03	0.00	−102.28	64.56
	Hackett all species	ACDC	−1.23	1.00	−165.90	22.71
		Brownian motion	**−0.82**	**1.00**	**−188.61**	**0.00**
		Ornstein–Uhlenbeck	−3.95	0.00	−116.21	72.40
	Hackett sequenced species	ACDC	−1.26	1.00	−146.66	20.08
		Brownian motion	**−0.91**	**1.00**	**−166.75**	**0.00**
		Ornstein–Uhlenbeck	−4.03	0.00	−102.28	64.46
Density	Ericson all species	ACDC	−1.20	1.00	−166.00	22.78
		Brownian motion	**−0.87**	**1.00**	**−188.78**	**0.00**
		Ornstein–Uhlenbeck	−3.48	0.00	−113.40	75.38
	Ericson sequenced species	ACDC	−1.21	1.00	−146.54	20.26
		Brownian motion	**−0.95**	**1.00**	**−166.80**	**0.00**
		Ornstein–Uhlenbeck	−3.44	0.00	−98.80	68.00
	Hackett all species	ACDC	−1.17	1.00	−165.75	22.82
		Brownian motion	**−0.80**	**1.00**	**−188.58**	**0.00**
		Ornstein–Uhlenbeck	−3.48	0.00	−113.40	75.17
	Hackett sequenced species	ACDC	−1.19	1.00	−146.47	20.23
		Brownian motion	**−0.89**	**1.00**	**−166.70**	**0.00**
		Ornstein–Uhlenbeck	−3.44	0.00	−98.81	67.89

Bold indicates best-fit model, that is, smallest Akaike information criterion; AIC (Ericson *et al.,* 2006; Hackett *et al.*, 2008).

with the complex punctuated evolution reported by Rutkowska *et al.* (2012) and Zhou *et al.* (2014). Our finding of an abrupt shortening of W chromosome length suggests that the key to understanding sex chromosome evolution may not rest with the gradual suppression of recombination.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. W/Z chromosome size ratios from karyotypes, also listing the number of substitutions per site from Hackett *et al.* (2008) and sources for karyotypes (references for Table S1). Table S1 provides W/Z data in addition to that used in Rutkowska *et al.* (2012).

SHARED DATA

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.bm562 (Gorelick *et al.*, 2016).