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The long and the short of avian W chromosomes: no evidence for gradual W shortening

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The well-established view of the evolution of sex chromosome dimorphism is of a gradual genetic and morphological degeneration of the hemizygous chromosome. Yet, no large-scale comparative analysis exists to support this view. Here, we analysed karyotypes of 200 bird species to test whether the supposed directional changes occur in bird sex chromosomes. We found no support for the view that W chromosomes gradually become smaller over evolutionary time. On the contrary, the length of the W chromosome can fluctuate over short time scales, probably involving both shortening and elongation of non-coding regions. Recent discoveries of near-identical palindromes and neo-sex chromosomes in birds may also contribute to the observed variation. Further studies are now needed to investigate how chromosome morphology relates to its gene content, and whether the changes in size were driven by selection.

Keywords: avian karyotype; sex chromosome; W degeneration

1. INTRODUCTION

The evolution of sex chromosome dimorphism (SCD) is generally thought to follow a repeatable pattern. If one of the sex chromosomes carries a sex-determining region with at least two loci that should be linked together, selection favours the process of preventing sex chromosomes from recombination [1,2]. In turn, the lack of recombination leads to degeneration of the chromosome that is present only in one sex. The genetic degeneration of the hemizygous chromosome can occur because of Hill–Robertson effects, such as Muller’s ratchet, background selection and the hitchhiking of deleterious alleles to advantageous mutations (reviewed in Charlesworth & Charlesworth [3]). Genetic degeneration, accompanied by morphological shrinking of hemizygous chromosomes, is observed in both XY and ZW chromosome systems [2,4]. Yet, most of the research focus has been on the mammalian Y chromosome [5], in which rapid degeneration can, to some extent, be attributed to a higher mutation rate in males (owing to more cell divisions in the germ line),

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and to a smaller effective population size of Y compared with X (reviewed by Ellegren [6]).

Parallel to what is observed in Y, the W chromosome in birds and snakes also seems to have degenerated over evolutionary time scales. In general, during the course of evolution, the W becomes morphologically small, with a higher proportion of heterochromatin, fewer genes and restricted areas of recombination [6]. However, in a ZW system one can separate the effect of the hemizygous chromosome from the male-biased mutations, providing an excellent system for understanding the evolution of SCD.

The avian Z chromosome is remarkably preserved across taxa [4]. Usually, it is 4–6th in size among all the chromosomes and has a stable absolute length (75 Mbp in the chicken *Gallus gallus*, 81 Mbp in the turkey *Meleagris gallopavo* and 73 Mbp in the zebra finch *Taeniopygia guttata*; www.ncbi.nlm.nih.gov). In chicken and zebra finch, Z has a conserved its gene content, although gene order has changed [7]. In contrast, the W is highly variable and its large portions are not shared among bird species [4]. In paleognathous birds, Z and W are similar to each other morphologically as well as at the molecular level [8]. In neognathous birds, the W is much smaller than the Z and its morphology and repetitive sequences differ between galliformes and passerines [9]. It has been commonly assumed that W degeneration over time is also characterized by a reduction in size. This belief originated from Ohno’s [10] initial recognition of SCD among snakes and birds and was further maintained by comparisons of W sizes among a handful of avian species [4,9]. The idea of morphological reduction of W is also enhanced by our perspective of the gradual shortening of human Y chromosome [5], although there are examples of Y becoming larger than X in non-human species [2]. However, there has been no systematic large-scale comparative analysis that would support the view of W shrinkage.

Here, we challenge the common belief regarding W shortening by studying SCD in species representing over 35 bird families. We assume that the size of Z is stable across the taxon [4,11]. If the commonly supposed pattern of directional changes in W is correct, closely related species should have very similar SCD. Furthermore, the faster evolutionary rate, approximated by the smaller body mass of the species (and thus shorter generation time, [12]), and by the number of species within a family [13], should correlate positively with SCD (for genome size effects, see the electronic supplementary material). Alternatively, it is plausible that during the course of evolution the genetic degradation of W was not always accompanied by its shrinkage, because the direction of change in length is influenced by the relative rates of deletions and insertions of genetic material [2]. If this is the case, we should observe a random pattern of SCD across birds.

2. MATERIAL AND METHODS

Data on chromosome morphology were obtained from published avian karyotypes. We performed literature searches on the Web of Science (the last search was conducted in August 2011), using the key words: ‘avian’ or ‘bird’ and ‘karyotype’ or ‘chromosome’. As older papers do not always appear in such searches, we also used ‘The list of karyological references’ [14]. We found 40 papers

containing good-quality photographs of karyotypes of 200 species. When the images were obtained from more than one source, we used the better quality one for analysis. To assess the SCD, we scanned the pictures, measured the length of the Z and W chromosomes and calculated the ratio of Z to W length. Species body masses (the mean values for both sexes) were obtained from Dunning [15], and genome sizes from <http://www.genomesize.com>. The number of species in a family was taken from Bennett & Owens [16], and the number in a subfamily from Dale *et al.* [17].

All statistical analyses were conducted in the R environment (v. 2.13.1; R Development Core Team). Phylogenetic generalized linear mixed-effects models (PGLMMs) were implemented using the function *MCMCglmm* [18]. We constructed a tree topology based on the avian supertree from Davis [19]. To test the hypotheses described above, we ran several PGLMMs with the Z to W ratio (ZW ratio; log-transformed for normality) as the response variable and the phylogeny as a random effect: (i) the intercept model to calculate phylogenetic signal in the ZW ratio ($n = 200$), (ii) the model with species body mass (log-transformed) as a fixed factor ($n = 188$), and (iii) the models with species numbers within avian families or subfamilies as fixed factors ($n = 189$ and $n = 200$, respectively).

3. RESULTS

As a measure of phylogenetic signal, we calculated phylogenetic heritability, H^2 *sensu* Lynch [20], which ranges from 0 (the response trait is free from phylogenetic relatedness) to 1 (the trait values among species are proportional to their phylogenetic relatedness; i.e. equivalent to Pagel's λ ; [21]). We found a very weak phylogenetic signal in the ZW ratio (PGLMM: $H^2 = 0.061$, 95% credible interval, CI = <0.0001 to 0.383). To put this H^2 -value into perspective, we also calculated a phylogenetic signal for species body mass—a trait well known for having a strong phylogenetic signal in birds [22]. As expected, we found a very strong signal (PGLMM: $H^2 = 0.988$, 95% credible interval, CI = 0.943–0.999; see electronic supplementary material, figure S2). The phylogenetic distribution of the ZW ratio is visualized in figure 1 (see also electronic supplementary material, figure S1). The weak phylogenetic signal of the ZW ratio (i.e. near random distribution of trait values) is apparent. The species body mass did not explain the variation in the ZW ratio (PGLMM: $b_{[\ln(\text{species mass})]} = -0.005$, 95% CI = -0.032 to 0.023). Furthermore, neither the number of species in the family nor in the sub-family accounted for the variation in the ZW ratio (PGLMM: $b_{[\text{species number}]} = 0.0002$, 95% CI = -0.0001 to 0.0004; $b_{[\text{sub-species number}]} = -0.0001$, 95% CI = -0.0005 to 0.0005). The full results of all PGLMMs are included in the electronic supplementary material.

4. DISCUSSION

Our results do not support the common assumption that the W chromosome has become steadily reduced over evolutionary time, nor that faster rates of evolution approximated by the smaller body mass of the species and by the number of species within a family correlate with increased SCD. On the contrary, we demonstrate that the length of the W can change over a very short time scale; that is, closely related species can have radically different SCD (figure 1). The observed variation in SCD among and within avian families could stem from two different processes acting on W chromosomes: the evolution of coding and of non-coding regions.

Despite the sequencing of two avian genomes (chicken and zebra finch), we still know very little

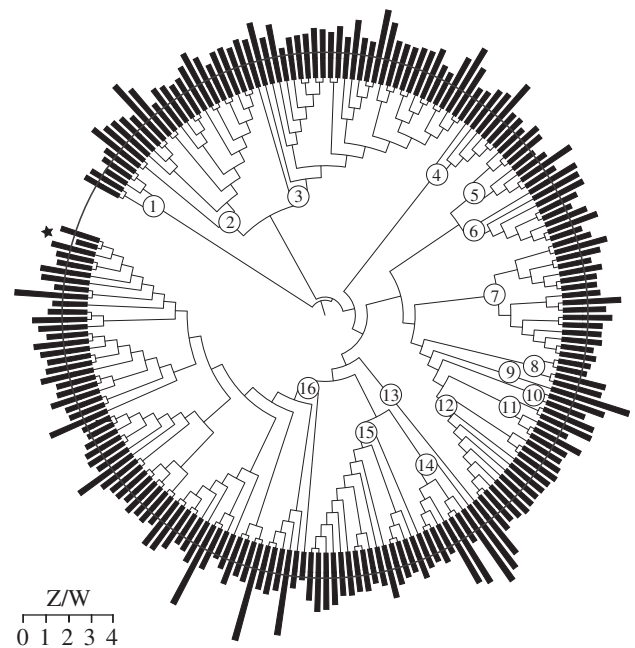


Figure 1. A phylogenetic distribution of the ZW ratio (Z/W) for 200 avian species. The Z/W ratio of 1 (indicated by a grey ring) reflects a lack of size dimorphism between the sex chromosomes. The numbers in circles represent bird orders: 1, Struthioniformes, Rheiformes, Tinamiformes (Paleognathae); 2, Galliformes; 3, Anseriformes; 4, Columbiformes; 5, Falconiformes; 6, Strigiformes; 7, Charadriiformes; 8, Gruiformes; 9, Podicipediformes; 10, Suliformes, Gaviiformes; 11, Pelecaniformes; 12, Ciconiiformes; 13, Trochiliformes; 14, Coraciiformes, Piciformes; 15, Psittaciformes; 16, Passeriformes. For species names and exact bar values, refer to electronic supplementary material, figure S1. The star indicates crimson finch (*Neochemia phaeton*).

about the gene content of the W chromosome in birds. The presence of some genes involved in the coding of female-specific traits [4] could constrain changes in W size, although the candidate sex-determining gene is located on the Z [23]. W elongation may happen if in species with high mutation rate essential genes were maintained as inverted repeats (i.e. near-identical palindromes; as found in New World sparrows and blackbirds [24]). Further studies should shed more light on the significance of W's genetic constitution.

As the greatest part of the W of neognathous birds consists of non-coding sequences, W elongation could result from expansion of those regions [2]. In our dataset (figure 1), the W of the crimson finch *Neochemia phaeton* is bigger than the Z, providing the most remarkable example of how heterochromatic addition might change chromosome morphology [25]. Both coding and non-coding regions of the W could get larger owing to fusion with the autosomal segments that create neo-sex chromosomes, such as those observed in *Drosophila* (reviewed in Bergero & Charlesworth [2]). Indeed, it has been recently reported that Z and W in warblers are the neo-sex chromosomes resulting from the fusion of the ancestral sex chromosome with the part of chromosome 4a [26]. See also the electronic supplementary material for analyses and discussion on Z size and genome size.

Our results show that SCD varies between closely related species. In fact, several reports of within-species

variation in sex chromosome morphology provide evidence for an on-going process of sex chromosome evolution. Specifically, different forms (varying in size and centromere position) of W were found in wheatears [27] and in grassfinches [25].

It is not known whether expansion of non-coding sequences and chromosome polymorphisms occur at random, or conform to any general pattern, or whether they are driven by natural selection. Selection that might affect SCD could involve genome size (see electronic supplementary material) or sexual dimorphism [28]. It has also been suggested that SCD facilitates offspring sex ratio manipulation [29]. More detailed investigations of coding and non-coding sequences located on the sex chromosomes should help to relate chromosome morphology and gene content.

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