

Parallel divergence and degradation of the avian W sex chromosome

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Sex chromosomes are ubiquitous in birds but our understanding of how they originated and evolved has remained incomplete. Recent work by Tsuda *et al.* on tinamou and ratite birds suggests that, although all bird sex chromosomes evolved from the same pair of autosomes, the Z and W sex chromosomes have diverged from one another several times independently. This parallel evolution of the avian W presents a means for comparison in studies of sex chromosome evolution, which could help us understand more about the general forces that shape the development of all types of sex chromosome.

Two basic types of sex chromosome

All mammals and many model organisms, including *Drosophila* and *Caenorhabditis elegans*, have male heterogametic sex chromosomes, where females are XX and males are either XY or XO. The sex chromosomes of flies, worms and mammals have arisen independently from one another, and comparisons among these systems have revealed much about the evolution of male heterogamety [1]. However, this is only half of the picture of sex chromosome evolution because many other animals, including birds, lepidopterans and some lizards and fish, are female heterogametic (where females are either ZW or ZO and males are ZZ) [2]. Birds are the most-studied female heterogametic clade, and our understanding of avian sex chromosome evolution has revealed important insights into female heterogamety [3,4], and into the evolution of sex chromosomes in general through comparisons with XY systems.

Extant birds can be divided into two ancient clades that split ~120 million years ago (Mya) [5]: the Palaeognathae comprises the ratites (ostriches, emus and allies) and tinamous; the Neognathae contains all other modern birds. Most sex chromosome research in birds has focused on the Neognathae, as this clade contains more species, including those with completed (chicken *Gallus gallus*) and nearly completed (zebra finch *Taeniopygia guttata*) genome sequences. All extant neognathous birds have highly differentiated sex chromosomes, with a large Z and a small and chiefly heterochromatic W, which derive from a single ancestral pair of autosomes [4,6]. We now know that the neognathous sex chromosomes follow the general pattern of sex chromosome evolution [7], with the initial cessation of recombination between the proto-sex chromosomes that

is extended in a stepwise fashion along most of the length of the gametologs (the two different sex chromosomes), eventually leading to divergence into recognizable Z and W chromosomes [8,9]. Recent work by Tsuda and colleagues [10] has expanded our understanding of avian sex chromosome evolution by revealing parallel processes of sex chromosome divergence between the Neognathae and the tinamou lineage of the Palaeognathae.

Parallel sex chromosome divergence in the Palaeognathae

Studies of palaeognathous sex chromosomes were previously confined primarily to ostriches *Struthio camelus* and emus *Dromaius novaehollandiae*, both members of the ratite lineage. These comparative cytogenetic studies established orthology between palaeognathous and neognathous Z and W chromosomes [6,11,12], and indicated that the ancestors of all modern birds had some version of a proto-Z chromosome that was passed on and retained in both daughter lineages after the avian divergence from reptiles [13]. However, ostriches and emus might present a biased picture of palaeognathous sex chromosomes, because the ratite Z and W have been maintained in an inexplicable arrested ancestral state. Homologous gene content in the ratite Z and W chromosomes, as well as cytological studies revealed that the emu and ostrich sex chromosomes are largely indistinguishable, recombine across most of their length and are only differentiated by a relatively small deleted region on the W [6,11]. Limited work in the tinamous [12] suggested that this ancestral state was not maintained in all palaeognathous species, but a detailed understanding of sex chromosome evolution across the Palaeognathae was lacking.

Tsuda and colleagues [10] have now shed much needed light on the evolution of the tinamou W chromosome, and have expanded our understanding of palaeognathous sex chromosomes beyond the ratites. Comparative cytogenetic and molecular sequence analysis in ostriches and the elegant crested tinamou *Eudromia elegans* has shown that, although the ratite gametologs are largely identical, the tinamou Z and W are not, and diverged from one another after the tinamou–ratite split ~80 Mya [5]. The process of tinamou W degradation could have been aided by the accumulation of repetitive DNA on the chromosome subsequent to the cessation of recombination [12]; however, the degree of differentiation in the tinamou sex chromosomes is not as significant as that in the Neognathae. Tsuda *et al.* also identified a small non-recombining region on the ostrich W,

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which could offer future insight into the mechanisms of avian sex determination, as well as the initial triggers of sex chromosome evolution [7]. What is most curious about this parallel divergence is the similarity of the tinamou and neognathous W. Despite some deviations in gene order owing to inversions, the non-recombining region of the

tinamou W retains some of the same genes as found on neognathous W [10,14] (Box 1) and, if this is not due to positional reasons, it could hint at some underlying adaptive reason, such as the need to maintain equal male and female gene expression for some loci.

Additionally, the new data reveal that the arrest of recombination between the avian proto-sex chromosomes originated on the q arm and has then, independently in the different avian lineages, progressed towards the centromere. In the Neognathae, the cessation of recombination has advanced beyond the centromere, and also covers a large portion of the p arm. However, whether the initial cessation of recombination that produced sex chromosome differentiation occurred in the ancient ancestor of the Neognathae and Palaeognathae, or occurred in both daughter lineages independently after they diverged, remains a mystery.

Implications of the tinamou W chromosome

The tinamou sex chromosomes are important for several reasons. Most significantly, they present a replicative natural laboratory for studying the evolution of female heterogamety, with which we can hope to differentiate those

Box 1. Parallel retention of W-linked genes

Genes in the non-recombining region of the W chromosome usually degrade and are eventually deleted or otherwise disappear [15]. It is a strange coincidence then that two genes have been retained on the non-recombining regions of both the tinamou and Neognathae W chromosome [10,14]. The gene encoding chromodomain helicase binding protein 1 (*CHD1*) has Z- and W-linked copies in the Neognathae and in the tinamou [10], and phylogenetic inference of this gene (Figure 1) suggests that the tinamou W and Z copies diverged from one another after the Palaeognathae–Neognathae split (~120 Mya [5]) and also the ratite–tinamou separation (~80 Mya [5]). This is supported by the fact that the ostrich *CHD1* maps to the region of the sex chromosome that is still recombining (i.e. there are no differentiated Z and W copies).

The same case holds for the spindlin (*SPIN*) gene [14], and there could well be more genes that have been retained on the non-recombining tinamou W chromosome that will come to light with further study. This parallel gene conservation on the W could prove important in helping elucidate the processes that influence whether genes are lost or retained during W and Y chromosome degradation.

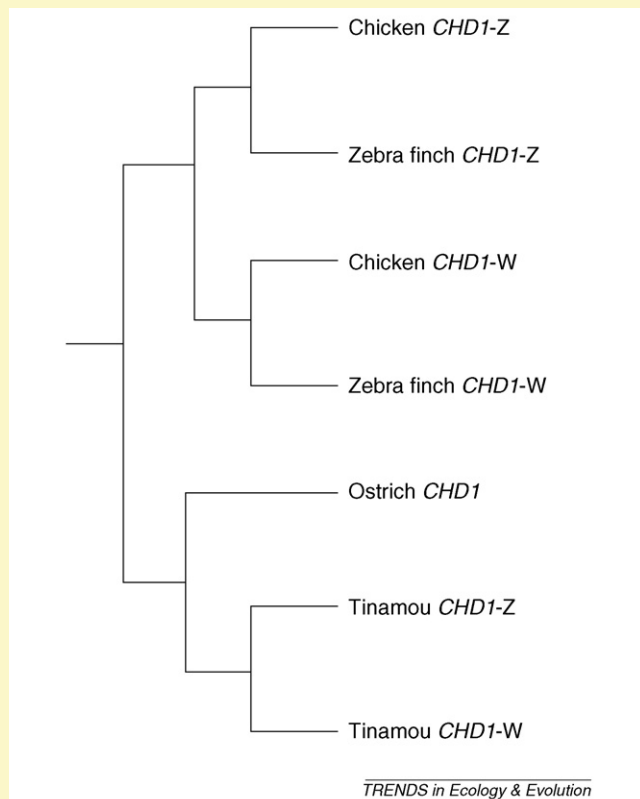


Figure 1. Phylogenetic reconstruction of avian Z and W gametologs of the gene encoding chromodomain helicase binding protein 1 (*CHD1*). Chicken *Gallus gallus* (GenBank NM204941 and AF181826) and zebra finch *Taeniopygia guttata* (GenBank NM001078178 and NM001078179), which diverged ~100 Mya [5], represent the Neognathae. Ostriches *Struthio camelus* (GenBank AB254867 and AB254868) and elegant crested tinamou *Eudromia elegans* (GenBank AB255123 and AB255124) are members of the Palaeognathae, and last shared a common ancestor ~80 Mya [5]. The Neognathae–Palaeognathae split occurred ~120 Mya [5]. The independent divergence of tinamou gametologs is suggested by the fact that the tinamou Z and W copies are more similar to each other than they are to both the ostrich and the respective neognathous coding sequences.

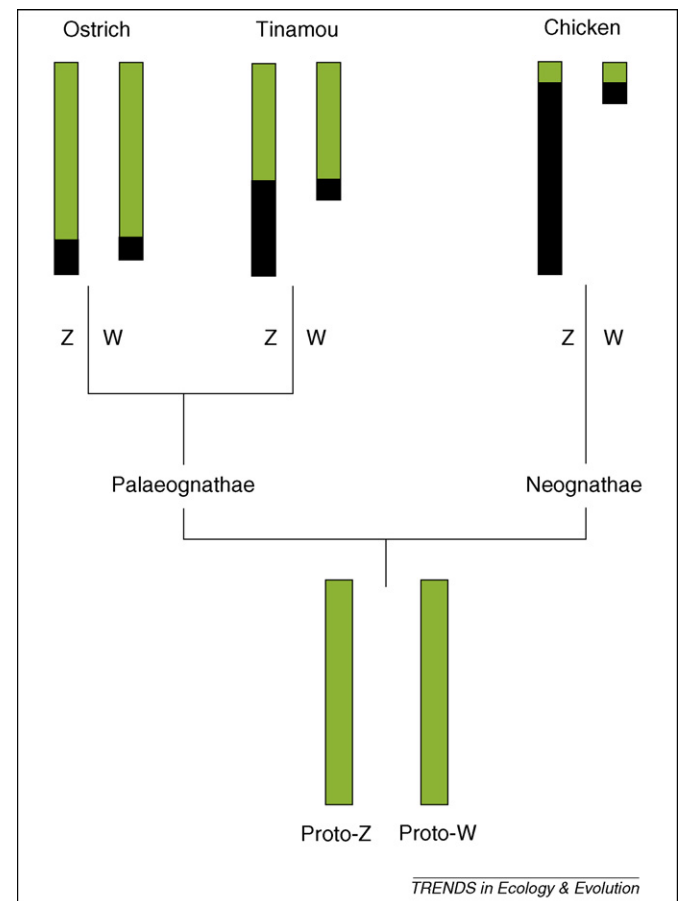


Figure 1. The progression of avian non-recombining regions. Ostriches represent the early stages of sex chromosome differentiation from an ancestral pair of sex chromosomes, with recombination (green bars) occurring along most of the length of the gametologs and only a small deletion differentiating the W from the Z. Since the divergence of tinamou and ostriches, recombination has ceased (black) over a larger portion of the tinamou gametologs, and more of the W chromosome has been eliminated, although the precise amount varies among tinamou species. The neognathous Z and W only recombine in a confined pseudo-autosomal region, and the W has undergone a massive reduction compared with the Z.

aspects of avian sex chromosome evolution that are inevitable from those that are due strictly to chance.

Additionally, even though the palaeognathous sex chromosomes are as old as those of the Neognathae, something has slowed the process of sex chromosome evolution in the group. This presents a living series of slow-motion time-shots in the progression of avian sex chromosomes, from the largely undifferentiated ostrich and emu Z and W, to the distinguishably different intermediate tinamou Z and W, to the terminal neognathous sex chromosomes that only recombine in a small and highly constrained pseudo-autosomal region (Figure 1). These characteristics offer a powerful clade for the study of sex chromosome evolution, which future sequence, linkage and cytogenetic analysis can exploit.

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Letters

Without morphology, cryptic species stay in taxonomic crypsis following discovery

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Recently, Bickford *et al.* [1] highlighted the importance of exploring cryptic diversity. Their biology-focused contribution is a reminder of the original questions regarding the current debate between molecular and traditional taxonomy [2], and a call for synergies between these approaches. Only an integration of all disciplines can promote biological research at the tempo set by the biodiversity crisis [3,4].

But one point is left unemphasized: the undiminished relevance of morphology-based alpha taxonomy (MOBAT), which is still the most important discipline for assigning

taxonomically valid names on the basis of name-bearing specimens (types). Types often date back to Linnaeus' time and are frequently unsuitable for molecular studies, despite progress in this field [5], even setting aside that museum curators usually refuse molecular sampling of fragile type specimens. MOBAT can link cryptic species to Linnean nomenclature and to established biological knowledge. Once discovered, many cryptic species can be identified by means of external physical characters [6], especially with methods of morphometric statistics [7].

Badly under-resourced [8], MOBAT cannot keep pace with the discovery of cryptic species, as illustrated by a

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