

# Triploid plover female provides support for a role of the W chromosome in avian sex determination

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Clemens Küpper<sup>1,2,\*</sup>, Jakob Augustin<sup>2,3</sup>,  
Scott Edwards<sup>1</sup>, Tamás Székely<sup>4</sup>,  
András Kosztolányi<sup>5</sup>, Terry Burke<sup>2</sup>  
and Daniel E. Janes<sup>1,†</sup>

<sup>1</sup>Museum of Comparative Zoology, Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA

<sup>2</sup>NERC Biomolecular Analysis Facility, Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK

<sup>3</sup>Department of Zoology, University of Gothenburg, PO Box 463, SE 405 30, Gothenburg, Sweden

<sup>4</sup>Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, UK

<sup>5</sup>Department of Ethology, Eötvös University, Pázmány Péter sétány, 1/c, 1117 Budapest, Hungary

\*Author for correspondence ([ckuepper@oeb.harvard.edu](mailto:ckuepper@oeb.harvard.edu)).

†Present address: National Institute of General Medical Sciences, National Institutes of Health, 45 Center Drive MSC 6200, Bethesda, MD 20892-6200, USA.

Two models, *Z Dosage* and *Dominant W*, have been proposed to explain sex determination in birds, in which males are characterized by the presence of two Z chromosomes, and females are hemizygous with a Z and a W chromosome. According to the *Z Dosage* model, high dosage of a Z-linked gene triggers male development, whereas the *Dominant W* model postulates that a still unknown W-linked gene triggers female development. Using 33 polymorphic microsatellite markers, we describe a female triploid Kentish plover *Charadrius alexandrinus* identified by characteristic triallelic genotypes at 14 autosomal markers that produced viable diploid offspring. Chromatogram analysis showed that the sex chromosome composition of this female was ZZW. Together with two previously described ZZW female birds, our results suggest a prominent role for a female determining gene on the W chromosome. These results imply that avian sex determination is more dynamic and complex than currently envisioned.

**Keywords:** sex determination; birds; microsatellites; aneuploidy

## 1. INTRODUCTION

Birds show striking sexual dimorphism with pronounced phenotypic differences between males and females. Sex in birds is determined genetically; males are ZZ and females are ZW. However, precisely how the phenotypic sexual dimorphism is initiated, is debated [1–3]. Two models have been proposed to explain sex determination in birds [4]. The *Z Dosage* model postulates that the

main determinant for sex is located on the Z chromosome. This sex determinant interacts with an autosomal gene and, depending on the ratio between copies of Z chromosomes and autosomes (*Z:A* ratio), the embryo develops as male or female. *Z Dosage* is based on the observed ineffective dosage compensation for Z genes, i.e. their expression is proportional to the copy number in birds [5,6]. The model is supported by experimental RNA inhibition of the Z-linked *DMRT1* gene, a major sex determinant in vertebrates [2]. When *DMRT1* was inhibited early in development, ZZ chicken *Gallus gallus* embryos subsequently developed ovaries but no testes. By contrast, the *Dominant W* model postulates that the main determinant for females is located on the W chromosome. For example, the presence of a gene located on the W chromosome may antagonistically interact with *DMRT1* by altering methylation of the male hypermethylated region adjacent to *DMRT1* in chicken [1]. However, such a ‘female gene’ has yet to be described in birds.

Chromosomal aberrations such as aneuploidy can help to clarify the sex determination mechanism although they are often already lethal at the embryonic stage in birds [7]. Triploid chickens with an ZWW genotype are not viable, whereas triploid ZZZ chickens develop a male phenotype but produce only abnormal sperm. Triploid ZZW chickens initially develop female phenotypes but before sexual maturity they develop male phenotypes [8]. Importantly, these intersexual chickens fail to produce viable gametes [8].

Here, we report a female putative triploid Kentish plover *Charadrius alexandrinus* that reproduced successfully in a natural population. We explore the type of its sex chromosome aneuploidy and discuss the implications of this case for models of avian sex determination.

## 2. MATERIAL AND METHODS

The female in question was a regular breeder captured during incubation in 1997 and 1999 at Tuzla, Turkey (36°42' N, 35°03' E). The first clutch in 1997 was predated, but in 1999 the entire clutch hatched and all family members were sampled for blood. Twenty-five microlitres of blood were taken from either the brachial vein (adults) or metatarsal vein (chicks) and stored in Queen's lysis buffer [9]. The female and her mate were sexed in the field based on plumage characteristics and sex-specific pattern of incubation in this species [10–12]. Molecular sexing using P2/P8 primers to amplify W- and Z-specific chromohelicase DNA binding protein fragments [13] confirmed the phenotypic sexing results of adults and showed that all three chicks were male. The family was genotyped using 33 microsatellite markers, including two Z-linked and one W-linked locus [14–17]. Genotypes were checked for consistency across two runs. Because no shorebird genome is yet available we mapped microsatellite locations to the chicken (WSHUC2) and zebra finch *Taeniopygia guttata* (taeGut3.2.4) genome databases following [16].

The three sex-linked markers (two Z-linked and one W-linked) had low polymorphism and the female was monomorphic at all of them (electronic supplementary material, S1). Therefore, we performed a peak height ratio analysis to establish composition and number of sex chromosomes [18]. We amplified products for W-linked *Calex-31* and Z-linked *Calex-26* together in a single PCR with 35 cycles and established the W/Z peak height ratio of the putative triploid female and 22 females from the same population that had the same genetic profiles at the sex-linked markers. We then compared the W/Z peak height ratio of the female in question to those of the control females.

## 3. RESULTS

For 17 of the 33 markers, we identified homologues on nine zebra finch and nine chicken chromosomes (electronic supplementary material, S1). The female had

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2012.0329> or via <http://rsbl.royalsocietypublishing.org>.

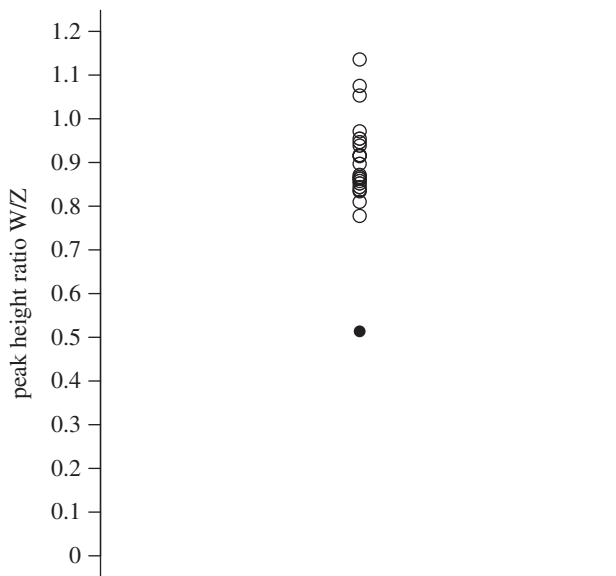


Figure 1. Peak height ratio of one putative triploid (black circle) and 22 diploid (open circles) females for *Calex-26* (Z-linked) and *Calex-31* (W-linked).

triallelic genotypes at 14 markers and all three maternal alleles were represented in the offspring at six markers (electronic supplementary material, S1; for example, electronic supplementary material, S2). Eight triallelic markers were mapped to six zebra finch and eight chicken autosomes. All alleles of the chicks were assigned to their social parents. Neither of the chicks nor the male showed triallelic genotypes. The peak height ratio analysis revealed that the triploid female differed from the mean peak height ratio of the 22 control females by 4.47 s.d. The W product was under-represented and reached only 45–66% of the ratio of the control females, consistent with an ZZW sex chromosome aneuploidy (figure 1).

#### 4. DISCUSSION

Triploidy is usually lethal at the embryonic stage in birds [7]. We report a triploid ZZW Kentish plover that behaved as a female and produced viable diploid offspring in the wild.

The Z:A ratio is an important feature of the Z Dosage model [4]. Triploid ZZW chickens that have an intermediate Z:A ratio of 2:3 are sex changers that start as females but assume phenotypic characteristics of males before reaching sexual maturity. In contrast to our plover female these chicken sex changers do not produce viable gametes [8]. During a period of 3 years, we observed two reproduction attempts of this female with the same male. The age of the female was at least 3 years when it reproduced successfully and was last seen alive. We consider it unlikely that she changed her sex subsequently, long after onset of sexual maturity and successful reproduction.

The observation of a reproducing ZZW female has implications for avian sex determination. Despite the recent support for an important role of *DMRT1* in the sex determination cascade in a bird [2], an effect of a W-linked gene that triggers femaleness should not be discarded [3]. This still unknown gene could

antagonistically interact with *DMRT1*, for example, through changes of methylation patterns [1]. In amphibians with a ZW sex determination system, *DM-W*, a recently identified truncated paralogue of *DMRT1* on the W chromosome, interacts antagonistically with *DMRT1* and is known to trigger femaleness [19]. *DM-W* has no known homologue in chickens, although the current lack of sequence information for the W chromosome from other birds does not rule out the presence of a *DMRT1* paralogue or other potentially female-determining genes in other avian lineages.

We suggest that more than one sex determination mechanism may have evolved in birds and that the current description of *DMRT1*-driven male determination in birds is incomplete or overly simplistic. In most vertebrate groups, the mechanism of sex determination is not fully conserved [20]. For example, switches between environmental and genetic sex determination (ZW or XY) have occurred frequently during the evolutionary history of reptiles [21–23]. Previously, two cases of adult ZZW females were reported in blue-and-yellow macaws *Ara ararauna* and great reed warblers *Acrocephalus arundinaceus* [24,25], two other non-galliform species. However, in both previous studies aneuploidy could not be established for the gonads. The females either did not have offspring [24] or transmitted only alleles of one Z chromosome to the offspring [25]. By contrast, we showed that the triploid plover female transmitted all three alleles to the offspring for at least six loci. Therefore, we conclude that her gonads were also triploid.

Observations of ZZW females exclusively in non-galliform birds suggest that an alternative sex determination mechanism may have evolved in this group. This is further supported by the large interspecific size variation of bird sex chromosomes [26], and expression differences of Z-linked genes between galliform and non-galliform birds [27]. Only recently, for example, a neosex chromosome was discovered through linkage analyses that arose from the fusion of the sex chromosomes with chromosome 4a in the warbler family *Sylviidae* [28]. Taken together, these reports suggest that avian sex determination is more complex and dynamic than currently recognized. We suggest that future studies should focus not only on chickens but also include a phylogenetically broad range of bird species to better understand the sex determination pathway in birds.

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