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# Instability of Sex-Determining Systems in Frogs

Michihiko Ito

## Abstract

All of the anuran amphibians examined so far have genetic sex-determining systems, which include female heterogametic ZZ/ZW and male heterogametic XX/XY types. For example, the Japanese wrinkled frog *Glandirana rugosa* has both types. Most of frog species including the African clawed frog *Xenopus laevis* possess homomorphic sex chromosomes, while most mammalian and avian species have heteromorphic sex chromosomes. Thus, there should be a variety of sex-determining genes and sex chromosomes in frogs, although only *X. laevis* W-linked gene *dm-W* has been reported as a sex-determining gene. Interestingly, estrogen or androgen can induce sex reversal in many frog species, suggesting a vital role of sex steroid hormones on sex identity. In other words, frogs in the same order are good examples for the understanding of diversity of sex-determining systems. In this chapter, I summarize the diversity of frog sex-determining systems and discuss why sex-determining genes and systems have been unstable in frogs.

**Keywords:** sex determination, sex chromosome, sex-determining gene, sex steroid, default sex, ectothermy

## 1. Introduction

Sexual reproduction is the most common life cycle in animals and plants. Meiotic recombination mediated through sexual reproduction is believed to allow genetic variation for survival of some populations against environmental changes. Thus, sex systems are very important for life evolution and biodiversity. In vertebrates, female and male sexes could be mainly defined by the property of gonads, ovaries producing eggs and testes producing sperm, respectively. Importantly, undifferentiated gonads in most vertebrate species have potential to differentiate into ovaries and testes. Then sex determination could be defined as the decision of bipotential gonads to develop as either ovaries or testes in vertebrates.

There are a variety of sex-determining systems in organisms. In vertebrates, they could be classified roughly into two types: genetic and environmental types. Endothermic vertebrates exclusively have the former system, which includes female (ZW) and male (XY) heterogametic sex chromosomes. Most mammalian and avian species have the XX/XY and ZZ/ZW systems, respectively, while there are both ZZ/ZW- and XX/XY-type systems in teleost fish, amphibians, and reptiles [1]. In addition, ectothermic vertebrates including reptiles and fish have not only the genetic sex-determining systems but also environmental sex-determining systems, such as temperature- and social-dependent types. Remarkably, all amphibian species possess the genetic systems, although they have ectothermic traits like reptiles and fish [1].

In the chapter, I introduce sex-determining systems, sex chromosomes, and sex-determining genes in amphibian frogs and discuss the relationships among them.

2. Sex-determining systems and sex chromosomes in frogs

As described in the above section, all anuran amphibians examined so far have the genetic sex-determining systems including the ZZ/ZW and XX/XY types (Table 1). For examples, the (African bullfrog) *Pyxicephalus adspersus*, African clawed frog *Xenopus laevis*, and the cane toad *Bufo marinus* have the ZZ/ZW type [2–5], while the African reed frog *Hyperolius viridiflavus* and the marsupial frog *Gastrotheca riobambae* adopt the XX/XY-type systems [6, 7]. Remarkably, the Japanese frog *Glandirana (Rana) rugosa* have five populations in Japan; their sex-determining systems include two ZZ/ZW and three XX/XY types [8].

Species	Sex-determining type	Morphology of sex chromosomes	Sex-determining gene
<i>Xenopus laevis</i> (African clawed frog)	ZZ/ZW	Homomorphic	W-specific <i>dm-W</i>
<i>Pyxicephalus adspersus</i> African bullfrog)	ZZ/ZW	Heteromorphic	
<i>Glandirana rugosa</i> (Japanese wrinkled frog)	ZZ/ZW or XX/XY	Heteromorphic/homomorphic	
<i>Gastrotheca riobambae</i>	XX/XY	Heteromorphic	
<i>Hyperolius viridiflavus</i> (African reed frog)	XX/XY	Homomorphic	

Table 1. Sex-determining systems, sex chromosomes, and sex-determining genes in frogs.

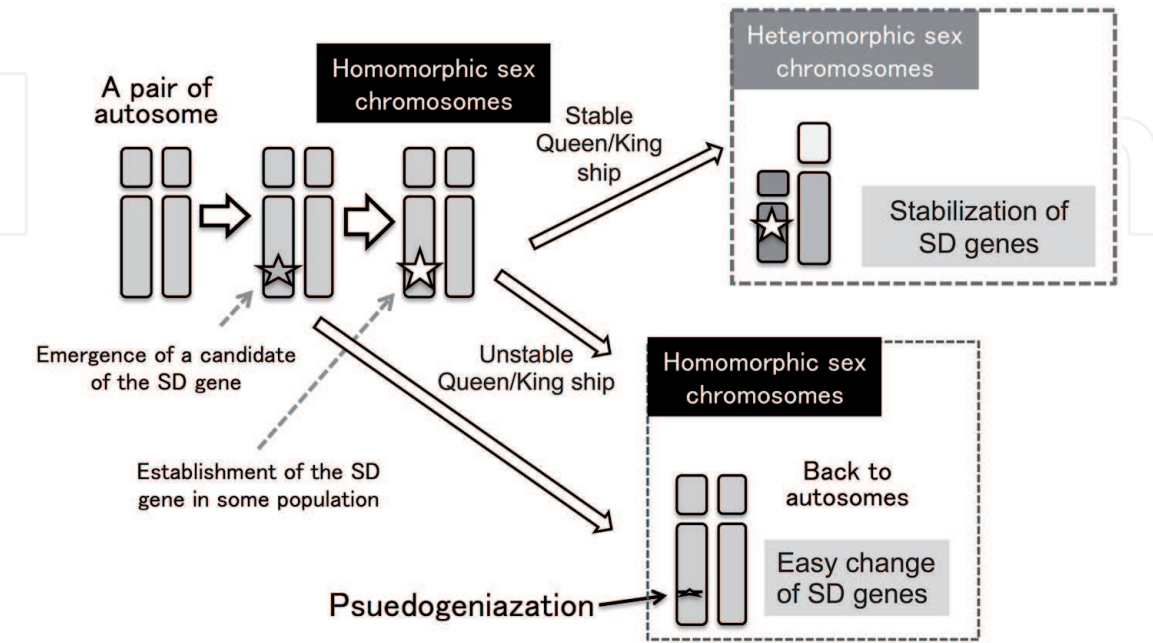


Figure 1. A model for emergence and evolution of sex-determining genes and homomorphic and heteromorphic sex chromosomes in vertebrates. The model includes a proposal of “GENE-eat-GENE” model for changes of sex-determining genes in homomorphic sex chromosomes.

The XX/XY and ZZ/ZW systems in most mammals and all birds examined have been maintained for more than a 100 million years, which is greatly connected with the monophyletic and heteromorphic sex chromosomes among most species of therian mammals or avians: the monophyly of the Z or Y sex chromosomes is closely related to the maintainability of the sex-determining gene *Dmrt1* on the Z chromosome or *Sry* on the Y chromosome, respectively [1]. In contrast, more than 90% species of frogs including *X. laevis* have homomorphic sex chromosomes [9–11]. In fact, sex chromosome homomorphism is well conserved among many vertebrate species except for mammals and birds. In 2012, we proposed a hypothesis for the coevolution of sex chromosomes and sex-determining genes, in which homomorphic sex chromosomes easily allow changes of sex-determining genes, resulting in changes of sex chromosomes. On the contrary, highly differentiated heteromorphic sex chromosomes including mammalian XY and avian ZW chromosomes are easily maintained, resulting in a stable fixation of a particular sex-determining gene, because each sex chromosome has gained important functions except for sex determination ([12, 13]; **Figure 1**). This context could lead to the conclusion that there are a variety of sex-determining genes in frogs [1], although few amphibian sex-determining genes except for *dm-W* we discovered in *X. laevis* [14] have been identified yet.

### 3. Discovery of a female sex-determining gene *dm-W* in the African clawed frog

In 1990, human *SRY* was discovered as a sex-determining gene, which was the first report among vertebrate species [15], followed by mouse *Sry* [16]. Now *Sry* is believed to be a sex-determining gene in many species of therian mammals. After about 10 years, the second vertebrate sex-determining gene named *dmy* (also known as *dmrt1bY*) was reported in the teleost fish medaka *Oryzias latipes* [17, 18]. Both the two genes function as Y-linked male-determining genes in the XX/XY-type sex-determining systems. In 2008, we discovered a W-linked sex (female)-determining gene *dm-W* from the frog *X. laevis* having a ZZ/ZW type [5]; *dm-W* was the first report as the sex-determining gene among amphibian species or ZZ/ZW-type vertebrate species. Among sex-determining genes reported so far, the *dm-W* gene is unique in that the gene is female genome-specific (W-linked) and causes ovary formation [5, 19]. Both the *dmy* and *dm-W* genes emerged from the duplication of *dmrt1* independently during species diversity in genus *Oryzias* and *Xenopus*, respectively [12]. Next, Smith et al. (2010) reported that the Z-linked *dmrt1* gene is necessary for male sex determination in the chicken (*Gallus domesticus*) [20]. Here I should describe what protein is doublesex Mab-3-related transcription factor 1 (DMRT1). The protein including a DNA-binding domain, called “DM domain,” functions in gonadal somatic cell masculinization and germ cell development in most vertebrates as transcription factors [21].

*X. laevis* is an allotetraploid species, whose ancestor might emerge by hybridization between two closely related *Xenopus* diploid species [22]. Therefore, there are two homoeologous L and S subgenome-derived genes in most of the genes in *X. laevis*. Partial duplication of S subgenome-derived *dmrt1* (*dmrt1.S*) leads to the emergence of *dm-W* [5, 23, 24]. In addition, we recently reported that *dm-W* evolved after allotetraploidization [24].

The DM domain of DM-W has about 90% amino acid sequence identity with those of DMRT1.L and DMRT1.S. However, the DM-W C-terminal region shares almost no similarity with those of DMRT1s. The last fourth exon of *dm-W* coding the C-terminal region emerged as a new exon [5]. We reported that DM-W and DMRT1 could cause primary ovarian and testicular formation in developing ZW



and ZZ gonads, respectively [19], and proposed a sex-determining model for the ZZ/ZW type that DM-W determines female sex by antagonizing DMRT1; *dm-W* evolved from a masculinizing gene *dmrt1* as a dominant negative-type gene [14].

#### 4. Sex reversal and sex chromosome differentiation

Although all frog species might genetically determine sex as mentioned above, most frog species could accept male-to-female or female-to-male sex reversals by treatment of sex steroids, estrogen, or androgen, respectively, during tadpole development [1]. Importantly, many frogs of them have homomorphic sex chromosomes. For example, *X. laevis* carries homomorphic W and Z sex chromosomes [5], and the estradiol-treated ZZ tadpoles developed to female adults [1]. In addition, we reported ZW female-to-male sex reversals in *X. laevis* transgenic tadpoles with *dm-W* knockdown or germline stem cell-specific knockdown of *dmrt1* and ZZ male-to-female sex reversals in *X. laevis* transgenic tadpoles carrying the *dm-W* expression plasmid [5, 19, 21].

Moreover, we recently analyzed detail structures of the sex chromosomes on 2Lq32-33 in *X. laevis*, revealing 278 kb W-specific region including three W-specific genes, the sex-determining gene *dm-W*, *scanw*, and *ccdc69w*, and 83 kb Z-specific region including one Z-specific gene *capn5z* [24]. Importantly, both gynogenetic WW and estrogen-driven sex-reversed ZZ individuals could develop into normal fertile females [25, 26]. These findings suggest that the homomorphic W/Z sex chromosomes in *X. laevis* are now differentiating but not so differentiated yet. In other words, *X. laevis* sex chromosomes have the potential to accept sex reversal and a new sex-determining gene.

#### 5. Conclusions and perspective

All frogs examined possess genetic sex-determining systems, and most of them have homomorphic sex chromosomes. The genetic systems could be easy to change during species diversity, that is, the instability of the systems, maybe because of homomorphic sex chromosomes, which could have a potential to convert a sex-determining gene into a new one on another chromosome, resulting in the change of sex chromosomes. Then I propose a “GENE-eat-GENE” model for turnover of sex-determining genes: there has been battles among the present sex-determining gene and candidates of new sex-determining genes for king/queen ship in some populations holding homomorphic sex chromosomes (**Figure 1**). Accordingly, I predict that there are great many sex-determining genes in frogs, although only one *dm-W* has been identified as sex-determining genes. Frogs belong to the order Anura, which collects several thousands of species. Therefore they could be good examples for studying the relationships between sex-determining systems and species diversity.

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