

## NEWS AND COMMENTARY

Experimental sex chromosome evolution?

# Heritable artificial sex chromosomes in medaka: a leap towards real-time observation of sex chromosome evolution

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Studies of extant sex chromosomes provide strong evidence for their evolution from ordinary autosomes, fuelled by suppressed recombination in the sex-specific region, which leads to eventual degeneration of the nonrecombining chromosome (Charlesworth and Charlesworth, 2000). However, the early stages of sex chromosome evolution are still fairly enigmatic, even in species with ‘young’ sex chromosomes. Even the neo-Y chromosome of *Drosophila miranda* is >1 million years old (Bachtrog and Charlesworth, 2002), whereas ‘young’ sex chromosomes of fish are in excess of 10 million years old (for example, Tanaka *et al.*, 2007), so we can only bear indirect witness to their evolution. As reported in this issue, researchers in Japan have developed a more practicable alternative to time travel by creating the first vertebrate heritable artificial sex chromosomes in the medaka, *Oryzias latipes* (Otake *et al.*, 2010). In doing so, they open a new and exciting chapter in sex chromosome research.

Sex determination is one of the most fundamental stages of an organism’s life. One could be forgiven then for expecting sex determination and sex chromosomes to be highly conserved throughout evolution. Sex chromosomes have, however, evolved independently, multiple times, even in closely related animal and plant groups. Take for example fish and amphibians. Recent studies have shown that different sex chromosomes have evolved independently even within sticklebacks (*Gasterosteus aculeatus*, Peichel *et al.*, 2004) and medaka (Tanaka *et al.*, 2007). Even more astonishing, both male and female heterogametic sex chromosome systems (XX/XY and ZZ/ZW, respectively) have been discovered in one species of frog (*Rana rugosa*, Ogata *et al.*, 2008)! In spite of this variety, and unlike the majority of their mammalian or avian cousins, nearly all fish and amphibians have undifferentiated (homomorphic) sex chromosomes. If sex determination is such a

fundamental process, why is there so much diversity in these groups, and why are their sex chromosomes not differentiated (heteromorphic) like those of mammals and birds?

A leading hypothesis is that sex chromosomes in lower vertebrates have high turnover rates, which allow them to avoid the path to destruction that accompanies reduced recombination on the hemizygous (Y or W) chromosome (Schartl, 2004). According to this ‘high turnover model’, new sex-determining genes appear regularly on ordinary autosomes, most likely by transposition of an established master sex-determining gene, or occasionally through mutation on an existing autosome. The new sex chromosomes replace their predecessors before they have time to accumulate deleterious mutations or structural changes that fuel degeneration of the nonrecombining sex chromosome (Charlesworth and Charlesworth, 2000). Studies of extant sex chromosomes have provided indirect evidence in support of the high turnover model (for example, Peichel *et al.*, 2004). However, an intriguing alternative hypothesis has recently been proposed. In his ‘old wine in a new bottle’ model, Perrin (2009) argues a role for sex reversal, which can occur in these groups (including medaka), and recombination patterns, which depend on phenotypic rather than genotypic sex. Reversing sex and recombination patterns purges the accumulated deleterious mutations on the nonrecombining chromosome and counteracts the degeneration. Under Perrin’s model, ‘young’ homomorphic sex chromosomes are predicted to harbour ancient sex-determining genes.

The experiment by Otake *et al.* shows the plasticity of homomorphic sex chromosomes and provides direct and convincing support for the high turnover model of sex chromosome evolution in lower vertebrates. With meticulous experimental procedures and high-

throughput screening of progeny, they showed that autosomes can quite easily become sex chromosomes—at least in the laboratory! It seems quite plausible that turnover also occurs in the wild, but can it explain the observation of homomorphic sex chromosomes in nearly all fish and amphibians? The relative importance of the ‘high turnover’ and ‘old wine in a new bottle’ models in governing sex chromosome evolution certainly warrants further investigation.

Artificial (transgenic) sex chromosomes have been created previously in vertebrates to show the male-determining function of *SRY/sry* (mice, Koopman *et al.*, 1991) and *DMY* (medaka, Matsuda *et al.*, 2007), but neither of these strains was viable. *Sry* transgenic XX mice were sterile, probably because key genes needed for spermatogenesis were not included in the transgenic region (Koopman *et al.*, 1991). Previous attempts to generate *DMY* transgenic medaka showed testis differentiation with spermatogenesis, but low transmission rates, hence transgenic strains could not be established (Matsuda *et al.*, 2007). Otake *et al.* (2010) built on the work of Matsuda *et al.* (2007) by integrating fluorescent markers into the transgenic region. This allowed them to screen transgene-positive XX male fish and identify fry that had inherited, and were therefore good candidates for passing on the novel sex chromosome to their own offspring.

In wild-type medaka, the sex-determining region is found in linkage group (LG) 1. Sex-linkage analysis of the transgenic strains of Otake *et al.* indicated that exogenous *DMY* was located on LG5 in one strain, and LG23 in another (Otake *et al.*, 2010). Real-time PCR experiments confirmed that *DMY* is only expressed in the testes of transgenic strains, and that they have multiple copies of *DMY* and higher *DMY* expression levels than wild-type medaka. Moreover, transgenic *DMY* XX male fish are functionally equivalent to wild-type males; they have identical testes histology and are fully fertile. LG5 and LG23 are therefore functioning as novel sex chromosomes.

The big question is ‘what will happen next?’ Will the novel sex chromosomes begin a slippery path to reduced recombination and eventual degeneration and destruction? What will fuel this process—accumulation of transposable elements and/or inversions that disrupt recombination? Will sexually antagonistic genes be added to the sex-differential region and lead to selection for reduced recombination along the chromosome?

Or, will transgenic *DMY* jump from chromosome to chromosome to avoid the cascade of demise? Investigating these questions might only seem realistic with an infinite amount of research funding and time (the kind of proposal you come up with when asked about your dream experiment in a job interview), but actually with medaka as a model system, it is perhaps within the realms of reality. Medaka have a generation time of 2 months, and are easily maintained in the laboratory. Great steps have been taken in recent decades in experimental evolution. The investigation of sex-determination and sex chromosome evolution, as well as other fundamental organismic traits, might now be amenable to experimental evolution in vertebrates.

### Conflict of interest

The author declares no conflict of interest.

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