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Sex Determination: Primitive Y Chromosomes in Fish

Dispatch

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Recent analyses of the chromosomal regions that determine male development in sticklebacks and medaka have revealed several features associated with incipient Y chromosome evolution, including suppressed crossing over and the accumulation of repetitive DNA.

Determination of sexual identity by genes associated with highly differentiated sex chromosomes is often assumed to be the norm, given our familiarity with the X and Y chromosomes of mammals and model organisms such as *Drosophila*. Even within tetrapod vertebrates, however, there is a wide diversity of sex determination mechanisms, with many examples of species with genetic sex determination but microscopically similar X and Y chromosomes, and numerous cases of environmental sex determination [1]. There is an even wider range of sexual systems in teleost fishes, with examples of self-fertilizing hermaphrodites [2], sequential hermaphrodites [3], and environmental sex determination [1].

Even where sex is genetically determined, the mechanisms vary enormously, with clearly distinguishable sex chromosomes being very rare [1,4]. It is easiest to see the footprints of the evolutionary forces that drive the evolution sex chromosomes in cases where the divergence of X and Y chromosomes has not reached its limit, with no genetic recombination between X and Y chromosomes over most of their length and a lack of functional genes on the Y chromosome [5]. The comparative genetics of sex determination systems in fish species may thus yield important insights into the evolution of sex chromosomes.

Despite pioneering classical genetic studies of sex determination in fish such as the medaka, the guppy and the platyfish [1], it has been difficult to obtain detailed genetic information on sex chromosome organisation in these species. With modern genomic methods, however, it is now feasible, but laborious, to characterise the sex determining regions of fish genomes. Studies of chromosomal regions that determine male development in two unrelated groups of fish species, published recently in *Current Biology* [6,7], show the promise of this approach.

The first of these studies [6] concerns the three-spined stickleback, *Gasterosteus aculeatus*, which is really a cluster of distinct but closely related species. An association between sexual phenotype in sticklebacks and the gene coding for the enzyme isocitrate dehydrogenase (*Idh*) has long been known [8]. By chance, *Idh* was picked up by Peichel *et al.* [6] in a cDNA clone during a screen for microsatellite markers in *G. aculeatus*, allowing access to the sex determining region. Amplification of a portion of the gene, using the polymerase chain reaction (PCR), revealed a band length difference strongly associated with sex in two independent crosses involving members of different species (the putative Y chromosome allele is 31 basepairs shorter than its X-linked homologue). Genetic maps based on microsatellite markers showed that the sex determination locus is located at one end of linkage group 19. Comparison of the maps of males and females revealed that crossing over in males is strongly suppressed over a wide region near the end of this chromosome: a pair of markers separated by 16-20 centiMorgans (cM) in females is only 1.5–3.5 cM apart in males (Figure 1).

Similar local suppression of crossing over occurs near the sex-determining regions of two other fish species, the guppy and the medaka [9,10]. Genes

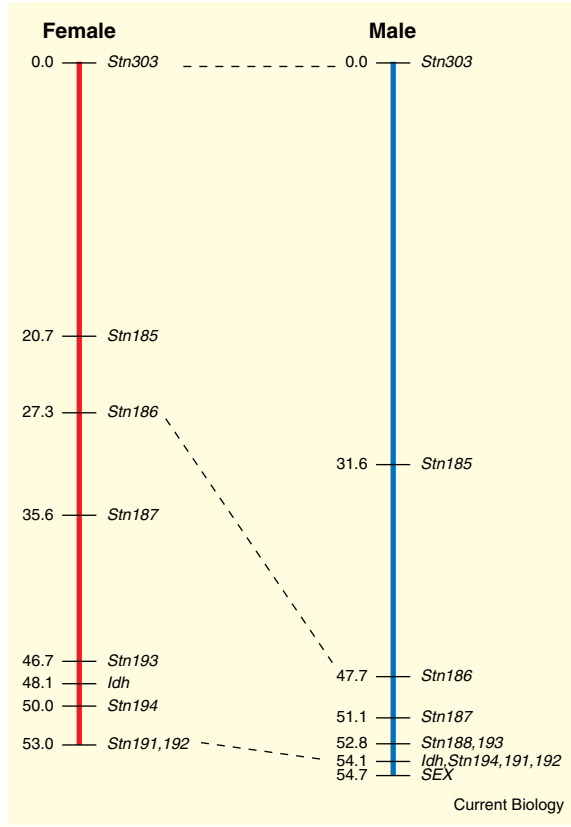

Figure 1. Genetic sex determination in sticklebacks.
Genetic maps of linkage group 19 of the three-spined stickleback in females and males, based on microsatellite markers in a cross between a lake species (Paxton, British Columbia) and a marine species from Japan. The location of the sex determining losus at the end of the chromosome, and the suppression of crossing over in this region in males, are easily seen. (Adapted from [6].)

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which are far from the sex-determining locus cross over freely between the 'proto-X' and 'proto-Y' chromosomes. The proto-X and proto-Y copies of genes that fail to cross over with the sex-determining locus are consequently genetically isolated from each other, and will diverge over evolutionary time. Suppression of crossing over between proto-X and proto-Y chromosomes near the sex-determining region is predicted by evolutionary models of the origin of sex chromosomes, which involve genes polymorphic for alleles with sexually antagonistic effects on fitness [1,11]. Restricted crossing over between the sex determining region and such genes reduces the frequencies of gametes with the 'wrong' alleles, and hence is favoured by selection [1,11].

The sex-determining 'locus' may itself be a compound 'supergene', with one gene that promotes female development on the proto-X chromosome but whose proto-Y allele inhibits it, and another gene with complementary effects on male development. This situation is predicted by models of the evolution of separate sexes from either hermaphroditism or environmental sex determination, and is supported by genetic studies of some plant sex determination systems [12,13]. Close linkage is required for the initial evolution of such a system, and further restriction of crossing over is expected to evolve subsequently. The exact nature of the sex-determination system in sticklebacks remains to be established, so that this possibility cannot be tested at present.

Peichel *et al.* [6] were able to characterise the stickleback sex-determining region in more detail by screening a bacterial artificial chromosome (BAC) library — derived from a mixture of males and females from a single population — for clones containing *Idh*. X- and Y-derived clones were identified on the basis of the sex difference in *Idh*. This yielded the complete DNA sequences of a continuous 250 kilobase region from both the proto-X and proto-Y chromosomes. This region contains four genes in addition to *Idh*, with no obvious role in sex determination. As might be expected from the suppression of crossing over in this region, there is extensive divergence between the X and Y sequences, with an average of only 64% identity per nucleotide site. The extent of divergence is, however, very variable across the region, with high X–Y similarity in coding sequences, but little similarity in many intergenic regions.

This variability in divergence probably results from insertions and deletions in regions where there is little functional constraint. These are especially likely to accumulate in the proto-Y sex-determining region, which is sheltered from selection by its heterozygosity and lack of recombination in males [5]. The Y region is 87 kilobases longer than its partner, which in part reflects the presence of more simple sequence repeats and duplications. In addition, it is enriched, relative to the X region, in transposable-element-derived sequences. Various population genetic processes can cause the accumulation of both duplicated sequences and transposable elements in regions of restricted crossing over [14], and such accumulation has been observed in several other examples of newly evolving Y chromosome systems [15–17].

Surveys of small samples of individuals from five populations showed no intra-population variability in the sequences of the *Idh* or *Znf* genes, but there was substantial divergence between samples from the Japan Sea compared with the Atlantic and Pacific Ocean. Nevertheless, there was strong clustering of sequences from the same sex chromosomes, even across different localities, showing that the divergence of the sex chromosomes must have preceded the divergence of these populations. The extensive divergence between these X- and Y-linked sequences suggests that the suppression of crossing over between the two chromosomes in males must be essentially complete in this small region. A similar pattern of localised suppression of crossing over associated with elevated divergence between X- and Y- linked sequences was reported recently for a plant, papaya [17]. At first sight, the stickleback result is hard to reconcile with the detection of several apparent crossovers between *Idh* and the sex determining gene [6]. Phenotypic sex in fish is notoriously labile, however, and these apparent recombinants may simply reflect occasional sex changes.

The related species *G. wheatlandi* shows no sign of any sex-specific variation at these two loci, despite having cytologically distinct X and Y chromosomes. This implies that the sex-determining regions must be distinct in these two taxa. It is possible that the sex-determination region of *G. aculeatus* may have evolved fairly recently, as a result of a male determining gene having been transposed onto linkage group 19 from elsewhere.

There is strong support for such an origin of the proto-Y chromosome in the medaka, *Oryzias latipes*, the other fish species which has recently been studied in detail at the molecular level. In this species — the first fish to be studied genetically — sex is genetically determined in the absence of true sex chromosomes, with males being heterozygous for a male-determining factor [1,10]. As in the stickleback, crossing over is strongly reduced near the sex-determining region in medaka [10]. Part of the male-specific chromosomal region containing the male determining factor has been cloned and sequenced. It contains a gene, *dmrt1bY* or *DMY*, which is required for male development [18,19].

Interestingly, medaka *dmrt1bY* is apparently homologous to *DMRT1* of mammals, which plays a major role in sex determination. *DMRT1* homologues are also implicated in sex determination pathways in other animals, including *Caenorhabditis elegans* and *Drosophila melanogaster*, as well as reptiles with environmental sex determination [20]. The male-specific region of the medaka proto-Y chromosome is enriched in repetitive sequences, and other genes included in it seem to have become non-functional [19]. It is only about 260 kilobases in length, representing about 1% of the length of the chromosome. There does not appear to be any X-linked homologue of *dmrt1bY*, but there is an autosomal homologue *dmrt1a*, with high sequence similarity.

Recent comparative studies suggest that the duplication of *dmrt1a* that generated *dmrt1bY* is of recent origin, as it is present in the close relatives of the medaka, *O. curvinotus* and *O. luzonenis*, but absent from *O. mekongensis* and more distant fish species [7]. There is no evidence that *dmrt1a* is a switch gene that decides sexual phenotype in these species. The duplication must have originated after the split between the lineages leading to *O. mekongensis* and the other species, and so must be 4–10 million years old. The evolutionary forces that favoured the establishment of this duplication and its new status as a male-determining gene, as well as the suppression of crossing over close to *dmrt1bY*, remain to be established. These studies of newly evolving sex-determining chromosomes in sticklebacks and the medaka have already provided some remarkably interesting results, and raise many new questions to be answered by further work.

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