Genetic basis of sex determination in sea lamprey

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ABSTRACT:

Genetic manipulation of sex determination is a powerful tool for control of pest species because introduction of modified individuals that produce highly male-biased offspring could eventually breed females out of existence. Because such sex-ratio distortion systems are especially effective once other control measures have already reduced pest abundance, genetic manipulation of sex ratios is being investigated as a complement to existing sea lamprey control. However, a genetic basis for sex determination in lampreys remains elusive. Environmental sex determination (ESD) has been suggested, but the evidence is equivocal, and no fish species with only ESD is known. Therefore, we hypothesized that there is a genetic basis to sex determination in the sea lamprey, even if it might be susceptible to environmental modification that would produce skewed sex ratios under some conditions. Previous studies in lampreys show that they lack heteromorphic sex chromosomes, and reduced-genome-representation genotyping (i.e., surveying only a portion of the genome) did not find any sex-specific differences. Because these previous approaches lack power to identify subtle differences, we conducted whole genome

resequencing on fin clips from 266 sea lamprey from populations where the upstream-migrant sex ratio was near parity to test for sex-specific differences in the genome. Because identifying “nascent” sex chromosomes with few sex-specific differences is challenging, often requiring specific combinations of methodologies, we developed a comprehensive workflow (SexFindR) to improve robustness and transparency in identifying sex-linked sequences at all levels of sequence divergence. Having validated our approach using publicly available data from five species that span the continuum of sex chromosome divergence—from homomorphic sex chromosomes with only one sex-specific single nucleotide polymorphism (SNP) to heteromorphic sex chromosomes with extensive differentiation—we applied SexFindR to our large-scale population genomics dataset for sea lamprey to decisively show that sea lamprey do not have sex-linked sequences in their somatic genome. Although it possible that sea lamprey exhibit ESD exclusively, another possibility is that sex is determined by the germline genome. Lampreys undergo a programmed genome rearrangement (PGR) during embryonic development that results in the deletion of ~20% of the genome from somatic cells. This means that the genome of the germ cells in the gonad retains ~500,000 bp that is absent from cells in all other parts of the body. Therefore, a second component to this project (which was not included in the original proposal) involved performing RNA sequencing and differential gene expression analysis on the gonads of 28 sea lamprey sampled across developmental stages to identify genes associated with both sex- and stage-specific gonadogenesis. In addition to identifying 2,617 genes in our transcriptomic analysis that had not been annotated in the sea lamprey genome before, we identified 638 germline-specific genes that exhibited a 36x greater odds of being expressed in testes than ovaries, including in prospective males several years before histological differentiation of the testis. Putative orthologs of some of these genes (e.g., fgf8/fgfr3, wnt, β catenin) have known functions in sex determination and differentiation in other vertebrates. These results suggest that low expression of the germline-specific genes results in a phenotypic female as the default sex, whereas high expression produces a male. We conclude that the germline-specific region (GSR) likely plays an important role in testicular differentiation. Although this means that sex-specific differences are absent from the somatic genome, thus making molecular sex identification from a fin clip or blood not possible, it may explain how this early vertebrate without heteromorphic sex chromosomes may combine environmental and genetic information to determine sex. We propose that in the undifferentiated gonad, in response to environmental cues (e.g., density, growth rate) and somatic-GSR molecular crosstalk, a decision is made to either open the chromatin of the GSR or let it remain silenced. If it remains silent, the gonad will initiate development of oocytes, while if the GSR is opened, a cascade of signaling events initiates development into a testis. Although it would require lethal sampling or gonadal biopsy, the gene expression pattern within the GSR could serve as an early indicator of future sex.