Development of methods of genotyping sex for use in endocrine disruption assays

Olmstead, Allen W//Lindberg-Livingston, Annelie//Woodis, Kacie K//Degitz, Sigmund J

Endocrine disrupting compounds have been shown to completely sex reverse both male and female individuals in amphibian, avian, fish, invertebrate, and reptile species. In many cases these sex-reversed individuals are morphologically indistinguishable from normal individuals. Detection of low-level sex reversal often requires large numbers of organisms to achieve the necessary statistical power, especially in those species with genetic sex determination and homomorphic sex chromosomes (such as amphibians and many fish). The usefulness of genotyping sex has been demonstrated in the Japanese medaka, where the sex-determining gene has been identified; however, in most aquatic toxicology model species, the sex-determining gene is unknown. We developed a genotyping method utilizing amplified fragment length polymorphisms (AFLP) in the amphibian, *Xenopus tropicalis*, for incorporation into endocrine disruptor screening assays that examine the effects of chemicals on gonad differentiation. AFLPs from 512 primer pairs were assessed in one spawn of *X. tropicalis.* Each primer pair yielded on average 100 fragments. In total 16 sex-linked AFLPs were identified, isolated, and sequenced. A recombination map of these AFLPs was generated using over 300 individuals with three AFLPs having a recombination rate of 0% with regard to sex. Cost-effective PCR methods were then developed that determine the presence of a given sex-linked marker for utilization in environmental toxicological assays. This approach to identifying sex-linked markers and developing sex genotyping methods is applicable to other species with genetic sex determination. Incorporation of sex genotyping in endocrine disruptor assays increases the statistical power of examining effects on gonad differentiation. Examples of data analysis from *X. tropicalis* toxicological studies where the genetic sex has been determined using these methods will be presented. This abstract does not necessarily reflect USEPA policy.