

**Molecular genetic studies of pollutant response in the
European flounder, *Platichthys flesus* (L.)**

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Abstract

Effects of man made pollutants on an ecosystem are initiated at the cellular level where a prime determinant for survival of an organism is its ability to metabolise and excrete toxic chemicals or their metabolites, thereby preventing cellular toxicity or damage to germ cell DNA. Cytochrome P450 (CYP) enzymes are responsible (in concert with the remainder of the *Ah* battery enzymes) for the metabolism of numerous xenobiotics and endogenous compounds, including the metabolic activation of most environmental toxic chemicals and carcinogens. Genetic polymorphisms which affect performance of these enzymatic detoxification systems may alter tolerance to pollutants and thus survival in polluted environments. Alterations in the susceptibility of individuals and the development of resistant populations has arisen by forced selection of populations with variant genes, resulting in increased detoxification capacity. There is evidence for such scenarios of variations in activities of pollutant biotransforming enzymes of fish contributing to survival in polluted estuarine environments and several chemically resistant populations have been identified in the USA and Europe. In fish it has been demonstrated that CYP1A enzyme activity is required to activate some carcinogenic xenobiotics to a metabolic state in which they can form DNA adducts. The mechanism of reduced CYP1A expression in highly contaminated populations may therefore represent resistance to chemical stressors. European flounder (*Platichthys flesus*) from some waterways which have a long history of severe sedimentary contamination do not show elevated levels of CYP1A. The aim of the current study was to investigate whether any heritable differences were apparent between offspring from parents

inhabiting long-term polluted and pristine areas. Flounder were obtained from a highly polluted estuary in the UK and crossed with fish from a relatively pristine environment. Offspring were raised in communal tanks in order to standardise environmental conditions, and allow investigations into the genetic variation of CYP1A. To allow identification of offspring to parental fish, polymorphic microsatellite loci were isolated and characterised for the flounder. Novel cDNA probes to transcription factors in the detoxification pathway (AhR2 and ARNT2) were cloned for flounder, and RT-PCR / Southern blot methods were developed for quantitation of gene transcript levels. A novel method of CYP1A quantification using real-time PCR was developed.

PAH and PCB exposure trials were carried out on mixed batch offspring, and CYP1A gene transcript levels assessed using Northern blot and real-time PCR techniques. Offspring were genotyped to their parents using the microsatellites obtained, and CYP1A transcript levels were correlated with clean and polluted areas. CYP1A was further correlated to transcription factor expression, and data are presented. Following exposure to the commercial PCB mixture, Aroclor 1254, CYP1A transcript levels were found to be significantly lower in families whose parents originated from a polluted area. This observation indicates that there is a possible genetic component to variation in CYP1A levels, and that these fish may have acquired a heritable tolerance to polluted areas. The lack of induction, or correlation with CYP1A levels, of AhR2 and ARNT2 expression indicates a possible AhR independent pathway for the metabolism of PCBs in the flounder.