**2007 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE (PNAS) PAPER SUMMARY**

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**Paper -**

David Crews, Andrea C. Gore, Timothy S. Hsu, Nygerma L. Dangleben, Michael Spinetta, Timothy Schallert, Matthew D. Anway, & Michael K. Skinner (2007) **Transgenerational Epigenetic Imprints on Mate Preference.** PNAS (In Press).

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**Abstract –**

Environmental contamination by endocrine-disrupting chemicals (EDC) can have epigenetic effects (via DNA methylation) on the germ line and promote disease across subsequent generations. In natural populations both sexes may encounter affected as well as unaffected individuals during the breeding season and any diminution in attractiveness could compromise reproductive success. Here we examine mate preference in male and female rats whose progenitors had been treated with the anti-androgenic

fungicide vinclozolin. This effect is sex-specific and we demonstrate that females three generations removed from the exposure discriminate and prefer males who do not have a history of exposure, while similarly epigenetically imprinted males do not exhibit such a preference. The observations suggest that the consequences of EDCs are not just transgenerational but can be ≥transpopulational≤, because in many mammalian species males are the dispersing sex. This indicates that epigenetic transgenerational inheritance of EDC action represent an unappreciated force in sexual selection. Our observations provide direct experimental evidence for a role of epigenetics as a determinant factor in evolution.

**Observation -**

The current study investigated animals prior to them developing disease to examine potential effects on mate selection. Sexual selection is a significant determinant in evolutionary biology. If all the progeny of an exposed individual are effected, environmental factors that influence behavior parameters such as mate selection could have a significant impact on evolution. The F3 control and EDC generations were examined with a series of mate selection behavior analyses. Interestingly, the control and EDC generation females preferred control generation males, while control and EDC F3 generation males had no preference. Therefore, an epigenetic transgenerational phenotype induced by an endocrine disruptor promoted an alteration in mate preference. This is one of the first experiments to document the ability of an environmental factor (i.e. endocrine disruptor) to promote an epigenetic change that influences a major determinant (i.e. sexual selection) in evolutionary biology.

A pregnant female was exposed to an environmental compound (i.e. endocrine disruptor) for a short period at a critical period of sex determination for the embryo. The male progeny later in life developed breast tumors, prostate disease, kidney disease, testis defects and immune abnormalities. This phenotype/disease state was passed to all subsequent generations examined. Only the original F0 generation mother was exposed to the environmental toxicant. Nearly all males of all generations had a disease state and passed it on to their progeny. Females developed disease, but could not pass the phenotype to the next generation. No known DNA sequence mutation mechanism can cause this type of transgenerational (i.e. heritable) disease phenotype. An epigenetic mechanism was identified in that the male germ-line (i.e. sperm) developed abnormal DNA methylation of specific genes and DNA sequences. The environmental toxicant permanently reprogrammed the sperm and induced new imprinted-like genes that passed the disease state on to all subsequent generations. Observations indicate that epigenetic mechanisms can permanently alter the germ-line and traits of progeny of an exposed individual and all subsequent generations.

**Impact -**

Evolutionary Biology:
Darwinian evolution is based on the appearance of genetic mutations (i.e. DNA sequence alterations) that promote a natural selection process and competitive biological advantage. This adaptation evolution process is the basis for our understanding of biology and the relationship of ecosystems. The concept that an environmental factor (i.e. toxicant) could induce an epigenetic effect that could promote a permanent reprogramming of the germ-line (i.e. sperm), impacts our concept of evolutionary biology. This suggests environmental impacts and epigenetics may be a critical variable in evolution. The current observations suggest new variables and factors in evolution that need to be considered and may explain some unexplained rapid evolutionary events previously observed.

Toxicology
Indicates that a class of environmental compounds known as endocrine disruptors can induce a permanent transgenerational effect on an individual. The exposure your pregnant grandmother had could induce a disease state in you and you will pass this on to your grandchildren. Therefore, the

potential hazard of environmental toxicants is dramatically increased, in particular for pregnant women in mid-gestation.

Molecular Basis of Epigenetic Heritable Traits and Disease
Previously we have realized that fetal and embryonic development events can impact disease states in the adult. A number of environmental toxicants have been shown after an embryonic exposure to cause an adult disease. The concept that these induced disease states could be transgenerational and permanently inherited has not been appreciated. Observations indicate that an epigenetic transgenerational mechanism could be involved in some heritable diseases. Many diseases have increased in frequency of occurrence but faster than can be explained from normal genetic (i.e. DNA sequence mutation) mechanisms. This epigenetic transgenerational phenomenon could explain the rapid onset of these diseases and would suggest an environmental factor in the process. This information provides new mechanistic insights into the molecular basis of disease and new therapeutic strategies to potentially treat the disease states. In addition, other areas of biology such as evolutionary biology are impacted with such an epigentic transgenerational mechanism.

**Summary -**

The transient exposure of a pregnant female at the time of embryonic sex determination to an environmental toxicant (endocrine disruptor) can induce an epigenetic transgenerational phenotype in subsequent generations. This has a significant impact on our understanding of how environmental factors can influence the genome and alter the potential evolution of the species. Sexual selection (i.e. mate preference) was found to be influenced which provides one of the first direct experiments to support a role for epigenetics in evolutionary biology.

**Bullet Points**

- Environmental factors (e.g. endocrine disruptors) can reprogram the germ-line (i.e. sperm) to influence heritable traits of all subsequent progeny.
- The epigenetic transgenerational phenotype suggests environment may influence biology through epigenetic alterations in genome activity.
- The current study suggests epigenetic transgenerational actions of environmental factors (endocrine disruptor) can influence a major determinant (i.e. sexual selection) in evolutionary biology.
- Supports an important role for epigenetics in evolutionary biology and provides an additional molecular mechanism to help understand evolutionary phenomenon.

**Epigenetics -**

Epigenetics does not involve DNA sequence changes, but factors around the genome that regulate genomic activity. An example is the chemical modification of the DNA (DNA Methylation). This can alter gene expression and determine if genes are turned on or off. A subset of genes called imprinted genes can transfer their epigenetic pattern, methylation of DNA, to the next generation and affect activity of DNA. Endocrine disruptors have been shown to modify a set of new imprinted-like genes. The frequency of an epigenetic effect is high compared to that of genetic sequence mutations.