Spring 2023 – Epigenetics and Systems Biology
Lecture Outline (Systems Biology)
Michael K. Skinner – Biol 476/576
Weeks 11 and 12 (March 2023)

Environmental Epigenetics

- Environmental Impacts on Biology
- Environment and Phenotype Variation
- Environmental Factors
- Environmental Epigenetics and Twin Studies
- Early life Exposures and Developmental Effects
- Nutrition and Epigenetics
- Environmental Toxicants and Epigenetics
- Environmental Induced Epigenetic Transgenerational Inheritance

Required Reading

Nilsson EE, Ben Maamar M, Skinner MK. Role of epigenetic transgenerational inheritance in generational toxicology. Environ Epigenet. 2022 Feb 16;8(1):dvac001. (PMID: 35186326)

Books (Reserve in Library)

Scott F. Gilbert and David Epel (2009) Ecological Developmental Biology. Sinauer Associates Inc. Sunderland, Massachusetts.

E-Book: Craig and Wong (2011) Epigenetics: A Reference Manual. Caister Academic Press. ISBN-13: 978-1904455882.

Literature

Gevaert AB, Wood N, Boen JRA, et al. Epigenetics in the primary and secondary prevention of cardiovascular disease: influence of exercise and nutrition. Eur J Prev Cardiol. 2022 Aug 22:zwac179. doi: 10.1093/eurjpc/zwac179. Online ahead of print.

Eid N, Morgan HL, Watkins AJ. Paternal periconception metabolic health and offspring programming. Proc Nutr Soc. 2022 May;81(2):119-125.

Holzapfel C, Waldenberger M, Lorkowski S, Daniel H; Working Group "Personalized Nutrition" of the German Nutrition Society. Genetics and Epigenetics in Personalized Nutrition: Evidence, Expectations, and Experiences. Mol Nutr Food Res. 2022 Sep;66(17):e2200077.

Guéant JL, Siblini Y, Chéry C, et al. Epimutation in inherited metabolic disorders: the influence of aberrant transcription in adjacent genes. Hum Genet. 2022 Jul;141(7):1309-1325.

Ryznar RJ, Phibbs L, Van Winkle LJ. Epigenetic Modifications at the Center of the Barker Hypothesis and Their Transgenerational Implications. Int J Environ Res Public Health. 2021 Dec 2;18(23):12728.
Diabankana RGC, Validov SZ, Vyshtakalyuk AB, Daminova A, Safin RI, Afordoanyi DM. Effects of Phenotypic Variation on Biological Properties of Endophytic Bacteria Bacillus mojavensis PS17. Biology (Basel). 2022 Sep 2;11(9):1305.
Aagaard A, Liu S, Tregenza T, Braad Lund M, Schramm A, Verhoeven KJF, Bechsgaard J, Bilde T. Adapting to climate with limited genetic diversity: Nucleotide, DNA methylation and microbiome variation among populations of the social spider Stegodyphus dumicola. Mol Ecol. 2022 Nov;31(22):5765-5783.
Low YW, Rajaraman S, Tomlin CM, et al. Genomic insights into rapid speciation within the world's largest tree genus Syzygium. Nat Commun. 2022 Sep 12;13(1):5031.
Hosseini S, Trakooljul N, Hirschfeld M, Wimmers K, Simianer H, Tetens J, Sharifi AR, Brenig B. Epigenetic Regulation of Phenotypic Sexual Plasticity Inducing Skewed Sex Ratio in Zebrafish. Front Cell Dev Biol. 2022 Jul 15;10:880779.
Chandana BS, Mahto RK, Singh RK, Ford R, Vaghefi N, Gupta SK, Yadav HK, Manohar M, Kumar R. Epigenomics as Potential Tools for Enhancing Magnitude of Breeding Approaches for Developing Climate Resilient Chickpea. Front Genet. 2022 Jul 22;13:900253.
Sharma M, Kumar P, Verma V, Sharma R, Bhargava B, Irfan M. Understanding plant stress memory response for abiotic stress resilience: Molecular insights and prospects. Plant Physiol Biochem. 2022 May 15;179:10-24.
Chénais B. Transposable Elements and Human Diseases: Mechanisms and Implication in the Response to Environmental Pollutants. Int J Mol Sci. 2022 Feb 25;23(5):2551.
Ke T, Tinkov AA, Skalny AV, Bowman AB, Rocha JBT, Santamaria A, Aschner M. Developmental exposure to methylmercury and ADHD, a literature review of epigenetic studies. Environ Epigenet. 2021 Nov 22;7(1):dvab014.
Andrawus M, Sharvit L, Atzmon G. Epigenetics and Pregnancy: Conditional Snapshot or Rolling Event. Int J Mol Sci. 2022 Oct 21;23(20):12698.
Rajamoorthi A, LeDuc CA, Thaker VV. The metabolic conditioning of obesity: A review of the pathogenesis of obesity and the epigenetic pathways that "program" obesity from conception.
Front Endocrinol (Lausanne). 2022 Oct 18;13:1032491.
Lapehn S, Paquette AG. The Placental Epigenome as a Molecular Link Between Prenatal Exposures and Fetal Health Outcomes Through the DOHaD Hypothesis. Curr Environ Health Rep. 2022 Sep;9(3):490-501.
Koemel NA, Skilton MR. Epigenetic Aging in Early Life: Role of Maternal and Early Childhood Nutrition. Curr Nutr Rep. 2022 Jun;11(2):318-328.
Brown AP, Cai L, Laufer BI, Miller LA, LaSalle JM, Ji H. Long-term effects of wildfire smoke exposure during early life on the nasal epigenome in rhesus macaques. Environ Int. 2022 Jan;158:106993.
Azoulay L, Bouvattier C, Christin-Maitre S. Impact of intra-uterine life on future health. Ann Endocrinol (Paris). 2022 Feb;83(1):54-58.
Kok DE, Richmond RC, Adriaens M, Evelo CT, Ford D, Mathers JC, Robinson N, McKay JA. Impact of In Utero Folate Exposure on DNA Methylation and Its Potential Relevance for Later-Life Health-Evidence from Mouse Models Translated to Human Cohorts. Mol Nutr Food Res. 2022 Feb;66(3):e2100789.

Lite C, Raja GL, Juliet M, Sridhar VV, Subhashree KD, Kumar P, Chakraborty P, Arockiaraj J. In utero exposure to endocrine-disrupting chemicals, maternal factors and alterations in the epigenetic landscape underlying later-life health effects. Environ Toxicol Pharmacol. 2022 Jan;89:103779.
Bermick J, Schaller M. Epigenetic regulation of pediatric and neonatal immune responses. Pediatr Res. 2022 Jan;91(2):297-327.
Sly P, Blake T, Islam Z. Impact of prenatal and early life environmental exposures on normal human development. Paediatr Respir Rev. 2021 Dec;40:10-14.
Antonelli MC, Frasch MG, Rumi M, Sharma R, Zimmermann P, Molinet MS, Lobmaier SM. Early Biomarkers and Intervention Programs for the Infant Exposed to Prenatal Stress. Curr Neuropharmacol. 2022;20(1):94-106.
Van Antro M, Prelovsek S, Ivanovic S, Gawehns F, Wagemaker NCAM, Mysara M, Horemans N, Vergeer P, Verhoeven KJF. DNA methylation in clonal Duckweed lineages (Lemna minor L.) reflects current and historical environmental exposures. Mol Ecol. 2022 Nov 2.
Mbiydzenyuy NE, Hemmings SMJ, Qulu L. Prenatal maternal stress and offspring aggressive behavior: Intergenerational and transgenerational inheritance. Front Behav Neurosci. 2022 Sep 23;16:977416.
Veronica CS, Ivan GM, Francisco GG. Evolutionary consequences of pesticide exposure include transgenerational plasticity and potential terminal investment transgenerational effects. Evolution. 2022 Sep 18. doi: 10.1111/evo.14613. Online ahead of print.
Lai KP, Tim Leung CC, Boncan DAT, Tam N, Lin X, Wang SY, Chan TF, Sun Wu RS, Chong Kong RY. Hypoxia-induced epigenetic transgenerational miRNAs dysregulation involved in reproductive impairment of ovary. Chem Biol Interact. 2022 Nov 1;367:110176.
Jeremias G, Veloso T, Gonçalves FJM, Van Nieuwerburgh F, Pereira JL, Asselman J. Multigenerational DNA methylation responses to copper exposure in Daphnia: Potential targets for epigenetic biomarkers? Chemosphere. 2022 Dec;308(Pt 1):136231.
Cao N, Lan C, Chen C, Xu Z, et al. Prenatal Lipopolysaccharides Exposure Induces Transgenerational Inheritance of Hypertension. Circulation. 2022 Oct 4;146(14):1082-1095.
Šrut M. Environmental Epigenetics in Soil Ecosystems: Earthworms as Model Organisms. Toxics. 2022 Jul 20;10(7):406.
Feiner N, Radersma R, Vasquez L, Ringnér M, Nystedt B, Raine A, Tobi EW, Heijmans BT, Uller T. Environmentally induced DNA methylation is inherited across generations in an aquatic keystone species. iScience. 2022 Apr 25;25(5):104303.
Beck D, Nilsson EE, Ben Maamar M, Skinner MK. Environmental induced transgenerational inheritance impacts systems epigenetics in disease etiology. Sci Rep. 2022 Apr 19;12(1):5452.
Yadav NS, Titov V, Ayemere I, Byeon B, Ilnytskyy Y, Kovalchuk I. Multigenerational Exposure to Heat Stress Induces Phenotypic Resilience, and Genetic and Epigenetic Variations in Arabidopsis thaliana Offspring. Front Plant Sci. 2022 Mar 28;13:728167.
Youssef NA. Potential Societal and Cultural Implications of Transgenerational Epigenetic Methylation of Trauma and PTSD: Pathology or Resilience? Yale J Biol Med. 2022 Mar 31;95(1):171-174.
Nilsson EE, Ben Maamar M, Skinner MK. Role of epigenetic transgenerational inheritance in generational toxicology. Environ Epigenet. 2022 Feb 16;8(1):dvac001.
Zhou R, Lu G, Yan Z, Jiang R, Sun Y, Zhang P. Epigenetic mechanisms of DNA methylation in the transgenerational effect of ethylhexyl salicylate on zebrafish. Chemosphere. 2022 May;295:133926.

Priyadarshini M, Ni JZ, Vargas-Velazquez AM, Gu SG, Frøkjær-Jensen C. Reprogramming the piRNA pathway for multiplexed and transgenerational gene silencing in C. elegans. Nat Methods. 2022 Feb;19(2):187-194.
Mohajer N, Joloya EM, Seo J, Shioda T, Blumberg B. Epigenetic Transgenerational Inheritance of the Effects of Obesogen Exposure. Front Endocrinol (Lausanne). 2021 Dec 16;12:787580.
Zhang Q, Tian Y. Molecular insights into the transgenerational inheritance of stress memory. J Genet Genomics. 2022 Feb;49(2):89-95.
Wellband K, Roth D, Linnansaari T, Curry RA, Bernatchez L. Environment-driven reprogramming of gamete DNA methylation occurs during maturation and is transmitted intergenerationally in Atlantic Salmon. G3 (Bethesda). 2021 Dec 8;11(12):jkab353.
Musolino E, Pagiatakis C, Serio S, Borgese M, Gamberoni F, Gornati R, Bernardini G, Papait R. The Yin and Yang of epigenetics in the field of nanoparticles. Nanoscale Adv. 2022 Jan 10;4(4):979-994.
Qu J, Wang Q, Sun X, Li Y. The environment and female reproduction: Potential mechanism of cadmium poisoning to the growth and development of ovarian follicle. Ecotoxicol Environ Saf. 2022 Oct 1;244:114029.
Li H, Zeng L, Wang C, Shi C, Li Y, Peng Y, Chen H, Zhang J, Cheng B, Chen C, Xiang M, Huang Y. Review of the toxicity and potential molecular mechanisms of parental or successive exposure to environmental pollutants in the model organism Caenorhabditis elegans. Environ Pollut. 2022 Oct 15;311:119927.
Murray KO, Clanton TL, Horowitz M. Epigenetic responses to heat: From adaptation to maladaptation. Exp Physiol. 2022 Oct;107(10):1144-1158.
Heindel JJ, Howard S, Agay-Shay K, et al. Obesity II: Establishing causal links between chemical exposures and obesity. Biochem Pharmacol. 2022 May;199:115015.
Cuomo D, Foster MJ, Threadgill D. Systemic review of genetic and epigenetic factors underlying differential toxicity to environmental lead (Pb) exposure. Environ Sci Pollut Res Int. 2022 May;29(24):35583-35598.
Pan J, Li X, Wei Y, Ni L, Xu B, Deng Y, Yang T, Liu W. Advances on the Influence of Methylmercury Exposure during Neurodevelopment. Chem Res Toxicol. 2022 Jan 17;35(1):43-58.
López de Las Hazas MC, Boughanem H, Dávalos A. Untoward Effects of Micro- and Nanoplastics: An Expert Review of Their Biological Impact and Epigenetic Effects.
Adv Nutr. 2022 Aug 1;13(4):1310-1323.
Vaudin P, Augé C, Just N, Mhaouty-Kodja S, Mortaud S, Pillon D. When pharmaceutical drugs become environmental pollutants: Potential neural effects and underlying mechanisms. Environ Res. 2022 Apr 1;205:112495.
Lagoa R, Marques-da-Silva D, Diniz M, Daglia M, Bishayee A. Molecular mechanisms linking environmental toxicants to cancer development: Significance for protective interventions with polyphenols. Semin Cancer Biol. 2022 May;80:118-144.
Grzybowski MW, Zwiener M, Jin H, Wijewardane NK, Atefi A, Naldrett MJ, Alvarez S, Ge Y, Schnable JC. Variation in morpho-physiological and metabolic responses to low nitrogen stress across the sorghum association panel. BMC Plant Biol. 2022 Sep 9;22(1):433.
Liu Y, Erbilgin N, Ratcliffe B, Klutsch JG, Wei X, Ullah A, Cappa EP, Chen C, Thomas BR, El-Kassaby YA. Pest defences under weak selection exert a limited influence on the evolution of height growth and drought avoidance in marginal pine populations. Proc Biol Sci. 2022 Sep 14;289(1982):20221034.
Aubry LM, Williams CT. Vertebrate Phenological Plasticity: From Molecular Mechanisms to Ecological and Evolutionary Implications. Integr Comp Biol. 2022 Oct 29;62(4):958-971.

Fu FX, Tschitschko B, Hutchins DA, Larsson ME, Baker KG, McInnes A, Kahlke T, Verma A, Murray SA, Doblin MA. Temperature variability interacts with mean temperature to influence the predictability of microbial phenotypes. Glob Chang Biol. 2022 Oct;28(19):5741-5754.
Ferrero-Serrano Á, Sylvia MM, Forstmeier PC, Olson AJ, Ware D, Bevilacqua PC, Assmann SM. Experimental demonstration and pan-structurome prediction of climate-associated riboSNitches in Arabidopsis. Genome Biol. 2022 Apr 19;23(1):101.
Linden B, Dalton DL, Van Wyk A, de Jager D, Moodley Y, Taylor PJ. Potential drivers of samango monkey (Cercopithecus albogularis) population subdivision in a highly fragmented mountain landscape in northern South Africa. Primates. 2022 May;63(3):245-260.
Yang CH, Fagnocchi L, Apostle S, Wegert V, Casaní-Galdón S, et al. Independent phenotypic plasticity axes define distinct obesity sub-types. Nat Metab. 2022 Sep;4(9):1150-1165.
Liu C, Wang Z, Hui Q, Goldberg J, et al. Association between depression and epigenetic age acceleration: A co-twin control study. Depress Anxiety. 2022 Jun 27. doi: 10.1002/da.23279. Online ahead of
Derakhshan M, Kessler NJ, Ishida M, et al. Tissue- and ethnicity-independent hypervariable DNA methylation states show evidence of establishment in the early human embryo. Nucleic Acids Res. 2022 Jun 17:gkac503.
Lundgren S, Kuitunen S, Pietiläinen KH, Hurme M, et al. BMI is positively associated with accelerated epigenetic aging in twin pairs discordant for body mass index. J Intern Med. 2022 Oct;292(4):627-640.
Wang Z, Hui Q, Goldberg J, Smith N, et al. Association Between Posttraumatic Stress Disorder and Epigenetic Age Acceleration in a Sample of Twins. Psychosom Med. 2022 Feb-Mar 01;84(2):151-158.
Li S, Ye Z, Mather KA, Nguyen TL, et al. Early life affects late-life health through determining DNA methylation across the lifespan: A twin study. EBioMedicine. 2022 Mar;77:103927.
King SE, Skinner MK. Epigenetic Transgenerational Inheritance of Obesity Susceptibility. Trends Endocrinol Metab. 2020 Jul;31(7):478-494.
Ben Maamar M, King SE, Nilsson E, Beck D, Skinner MK. Epigenetic transgenerational inheritance of parent-of-origin allelic transmission of outcross pathology and sperm epimutations. Dev Biol. 2020 Feb 1;458(1):106-119.
Duempelmann L, Skribbe M, Bühler M. Small RNAs in the Transgenerational Inheritance of Epigenetic Information. Trends Genet. 2020 Mar;36(3):203-214.
da Cruz RS, Chen E, Smith M, Bates J, de Assis S. Diet and Transgenerational Epigenetic Inheritance of Breast Cancer: The Role of the Paternal Germline. Front Nutr. 2020 Jul 15;7:93.
Nilsson EE, Ben Maamar M, Skinner MK. Environmentally Induced Epigenetic Transgenerational Inheritance and the Weismann Barrier: The Dawn of Neo-Lamarckian Theory. J Dev Biol. 2020 Dec 4;8(4):28.
Sarkies P. Molecular mechanisms of epigenetic inheritance: Possible evolutionary implications. Semin Cell Dev Biol. 2020 Jan;97:106-115.
Lewens T. Blurring the germline: Genome editing and transgenerational epigenetic inheritance. Bioethics. 2020 Jan;34(1):7-15.
Van Cauwenbergh O, Di Serafino A, Tytgat J, Soubry A. Transgenerational epigenetic effects from male exposure to endocrine-disrupting compounds: a systematic review on research in mammals. Clin Epigenetics. 2020 May 12;12(1):65.
Bautista NM, Crespel A, Crossley J, Padilla P, Burggren W. Parental transgenerational epigenetic inheritance related to dietary crude oil exposure in Danio rerio. J Exp Biol. 2020 Aug 20;223(Pt 16):jeb222224.

Yang Y, Jiang W, Yang S, Qi F, Zhao R. Transgenerational Inheritance of Betaine-Induced Epigenetic Alterations in Estrogen-Responsive IGF-2/IGFBP2 Genes in Rat Hippocampus. Mol Nutr Food Res. 2020 Apr;64(8):e1900823.
Woodhouse RM, Ashe A. How do histone modifications contribute to transgenerational epigenetic inheritance in C. elegans? Biochem Soc Trans. 2020 Jun 30;48(3):1019-1034.
Lempradl A. Germ cell-mediated mechanisms of epigenetic inheritance. Semin Cell Dev Biol. 2020 Jan;97:116-122.
Lismer A, Siklenka K, Lafleur C, Dumeaux V, Kimmins S. Sperm histone H3 lysine 4 trimethylation is altered in a genetic mouse model of transgenerational epigenetic inheritance. Nucleic Acids Res. 2020 Nov 18;48(20):11380-11393.
Monaco AP. An epigenetic, transgenerational model of increased mental health disorders in children, adolescents and young adults. Eur J Hum Genet. 2020 Sep 18.
Kaspar D, Hastreiter S, Irmler M, Hrabé de Angelis M, Beckers J. Nutrition and its role in epigenetic inheritance of obesity and diabetes across generations. Mamm Genome. 2020 Jun;31(5-6):119-133.
Ambeskovic M, Roseboom TJ, Metz GAS. Transgenerational effects of early environmental insults on aging and disease incidence. Neurosci Biobehav Rev. 2020 Oct;117:297-316.
Minow MAA, Colasanti J. Does variable epigenetic inheritance fuel plant evolution? Genome. 2020 May;63(5):253-262.
Ben Maamar M, Nilsson E, Thorson JLM, Beck D, Skinner MK. Transgenerational disease specific epigenetic sperm biomarkers after ancestral exposure to dioxin. Environ Res. 2021 Jan;192:110279.
Goldberg LR, Zeid D, Kutlu MG, et al. Paternal nicotine enhances fear memory, reduces nicotine administration, and alters hippocampal genetic and neural function in offspring. Addict Biol. 2021 Jan;26(1):e12859.
Harvey ZH, Chakravarty AK, Futia RA, Jarosz DF. A Prion Epigenetic Switch Establishes an Active Chromatin State. Cell. 2020 Mar 5;180(5):928-940.e14.
McCaw BA, Stevenson TJ, Lancaster LT. Epigenetic Responses to Temperature and Climate. Integr Comp Biol. 2020 Dec 16;60(6):1469-1480.
Shukla A, Yan J, Pagano DJ, et al. poly(UG)-tailed RNAs in genome protection and epigenetic inheritance. Nature. 2020 Jun;582(7811):283-288.
Wen Y, Rattan S, Flaws JA, Irudayaraj J. Multi and transgenerational epigenetic effects of di-(2-ethylhexyl) phthalate (DEHP) in liver. Toxicol Appl Pharmacol. 2020 Sep 1;402:115123.
Ben Maamar M, Beck D, Nilsson E, McCarrey JR, Skinner MK. Developmental origins of transgenerational sperm histone retention following ancestral exposures. Dev Biol. 2020 Sep 1;465(1):31-45.
Tikhodeyev ON. Heredity determined by the environment: Lamarckian ideas in modern molecular biology. Sci Total Environ. 2020 Mar 25;710:135521.
Neuparth T, Machado AM, Montes R, et al. Transgenerational inheritance of chemical-induced signature: A case study with simvastatin. Environ Int. 2020 Nov;144:106020.
Prokopec SD, Viluksela M, Miettinen HM, Boutros PC. Raimo Pohjanvirta R. Transgenerational epigenetic and transcriptomic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure in rat. Arch Toxicol. 2020 May;94(5):1613-1624.
Adrian-Kalchhauser I, Sultan SE, Shama LNS, et al. Understanding 'Non-genetic' Inheritance: Insights from Molecular-Evolutionary Crosstalk. Trends Ecol Evol. 2020 Dec;35(12):1078-1089.

Biwer C, Kawam B, Chapelle V, Silvestre F. The Role of Stochasticity in the Origin of Epigenetic Variation in Animal Populations. Integr Comp Biol. 2020 Dec 16;60(6):1544-1557.
Gangisetty O, Palagani A, Sarkar DK. Transgenerational inheritance of fetal alcohol exposure adverse effects on immune gene interferon-Ɣ. Clin Epigenetics. 2020 May 24;12(1):70.
Jawaid A, Jehle K-L, Mansuy IM. Impact of Parental Exposure on Offspring Health in Humans. Trends Genet. 2020 Nov 11;S0168-9525(20)30295-X.
Ben Maamar M, Beck D, Nilsson EE, Kubsad D, Skinner MK. Epigenome-wide association study for glyphosate induced transgenerational sperm DNA methylation and histone retention epigenetic biomarkers for disease. Epigenetics. 2020 Dec 9;1-18.
Nohara K, Suzuki T, Okamura K. Gestational arsenic exposure and paternal intergenerational epigenetic inheritance. Toxicol Appl Pharmacol. 2020 Dec 15;409:115319.
Senaldi L, Smith-Raska M. Evidence for germline non-genetic inheritance of human phenotypes and diseases. Clin Epigenetics. 2020 Sep 11;12(1):136.
Thorson JLM, Beck D, Ben Maamar M, Nilsson EE, Skinner MK. Epigenome-wide association study for pesticide (Permethrin and DEET) induced DNA methylation epimutation biomarkers for specific transgenerational disease. Environ Health. 2020 Nov 4;19(1):109.
Battaglia M. Epigenomic landscapes and their relationship to variation, fitness, and evolution. Neurosci Biobehav Rev. 2020 Feb;109:90-91.
Meyer DN, Crofts EJ, Akemann C, et al. Developmental exposure to Pb 2+ induces transgenerational changes to zebrafish brain transcriptome. Chemosphere. 2020 Apr;244:125527.
Barucci G, Cornes E, Singh M, et al. Small-RNA-mediated transgenerational silencing of histone genes impairs fertility in piRNA mutants. Nat Cell Biol. 2020 Feb;22(2):235-245.
Dumeige L, Nehlich M, Viengchareun S, et al. Preterm birth is associated with epigenetic programming of transgenerational hypertension in mice. Exp Mol Med. 2020 Jan;52(1):152-165.
Torres-Flores U, Hernández-Hernández A. The Interplay Between Replacement and Retention of Histones in the Sperm Genome. Front Genet. 2020 Jul 16;11:780.
Wu C, Sirard M-A. Parental Effects on Epigenetic Programming in Gametes and Embryos of Dairy Cows. Front Genet. 2020 Oct 14;11:557846.
Ben Maamar M, Nilsson EE, Thorson JLM, Beck D, Skinner MK. Epigenome-wide association study for transgenerational disease sperm epimutation biomarkers following ancestral exposure to jet fuel hydrocarbons. Reprod Toxicol. 2020 Dec;98:61-74.
Thorson JLM, Beck D, Ben Maamar M, Nilsson EE, McBirney M, Skinner MK. Epigenome-wide association study for atrazine induced transgenerational DNA methylation and histone retention sperm epigenetic biomarkers for disease. PLoS One. 2020 Dec 16;15(12):e0239380.
Tyebji S, Hannan AJ, Tonkin CJ. Pathogenic Infection in Male Mice Changes Sperm Small RNA Profiles and Transgenerationally Alters Offspring Behavior. Cell Rep. 2020 Apr 28;31(4):107573.
Jose AM. Heritable Epigenetic Changes Alter Transgenerational Waveforms Maintained by Cycling Stores of Information. Bioessays. 2020 Jul;42(7):e1900254.
Bertozzi TM, Elmer JL, Macfarlan TS, Ferguson-Smith AC. KRAB zinc finger protein diversification drives mammalian interindividual methylation variability. Proc Natl Acad Sci U S A. 2020 Dec 8;117(49):31290-31300.
Marrocco J, Einhorn NR, McEwen BS. Environmental epigenetics of sex differences in the brain. Handb Clin Neurol. 2020;175:209-220.

Hoyer-Fender S. Transgenerational effect of drug-mediated inhibition of LSD1 on eye pigment expression in Drosophila. BMC Ecol. 2020 Nov 23;20(1):62.
Liu J, Yu C, Doherty TM, Akbari O, Allard P, Rehan VK. Perinatal nicotine exposure-induced transgenerational asthma: Effects of reexposure in F1 gestation. FASEB J. 2020 Sep;34(9):11444-11459.
Wang X, Bhandari RK. DNA methylation reprogramming in medaka fish, a promising animal model for environmental epigenetics research. Environ Epigenet. 2020 Jul 7;6(1):dvaa008.
Spence JP, Lai D, Reiter JL, Cao S, Bell RL, Williams KE, Liang T. Epigenetic changes on rat chromosome 4 contribute to disparate alcohol drinking behavior in alcohol-preferring and -nonpreferring rats. Alcohol. 2020 Dec;89:103-112.
Kremsky I, Corces VG. Protection from DNA re-methylation by transcription factors in primordial germ cells and pre-implantation embryos can explain trans-generational epigenetic inheritance. Genome Biol. 2020 May 18;21(1):118.
Park HL. Epigenetic Biomarkers for Environmental Exposures and Personalized Breast Cancer Prevention. Int J Environ Res Public Health. 2020 Feb 13;17(4):1181.
Baugh LR, Day T. Nongenetic inheritance and multigenerational plasticity in the nematode C. elegans. Elife. 2020 Aug 25;9:e58498.
Schmitt C, Peterson E, Willis A, et al. Transgenerational effects of developmental exposure to chlorpyrifos-oxon in zebrafish (DANIO RERIO). Toxicol Appl Pharmacol. 2020 Dec 1;408:115275.
Martone J, Lisi M, Castagnetti F, et al. Trans-generational epigenetic regulation associated with the amelioration of Duchenne Muscular Dystrophy. EMBO Mol Med. 2020 Aug 7;12(8):e12063.
Johnson LM, Smith OJ, Hahn DA, Baer CF. Short-term heritable variation overwhelms 200 generations of mutational variance for metabolic traits in Caenorhabditis elegans. Evolution. 2020 Nov;74(11):2451-2464.
Rossnerova A, Izzotti A, Pulliero A, et al. The Molecular Mechanisms of Adaptive Response Related to Environmental Stress. Int J Mol Sci. 2020 Sep 25;21(19):7053.
Ramilowski JA, Yip CW, Agrawal S, et al. Functional annotation of human long noncoding RNAs via molecular phenotyping. Genome Res. 2020 Jul;30(7):1060-1072.
Ceribelli A, Selmi C. Epigenetic Methods and Twin Studies. Adv Exp Med Biol. 2020;1253:95-104.
Castelbaum L, Sylvester CM, Zhang Y, et al. On the Nature of Monozygotic Twin Concordance and Discordance for Autistic Trait Severity: A Quantitative Analysis. Behav Genet. 2020 Jul;50(4):263-272.
Turner JD, D'Ambrosio C, Vögele C, Diewald M. Twin Research in the Post-Genomic Era: Dissecting the Pathophysiological Effects of Adversity and the Social Environment. Int J Mol Sci. 2020 Apr 29;21(9):3142.
Battaglia M. Epigenomic landscapes and their relationship to variation, fitness, and evolution. Neurosci Biobehav Rev. 2020 Feb;109:90-91.
Filardi T, Panimolle F, Lenzi A, Morano S. Bisphenol A and Phthalates in Diet: An Emerging Link with Pregnancy Complications. Nutrients. 2020 Feb 19;12(2):525.
Woźniak E, Reszka E, Jabłońska E, et al. Glyphosate affects methylation in the promoter regions of selected tumor suppressors as well as expression of major cell cycle and apoptosis drivers in PBMCs (in vitro study). Toxicol In Vitro. 2020 Mar;63:104736.
Treviño LS, Dong J, Kaushal A, et al. Epigenome environment interactions accelerate epigenomic aging and unlock metabolically restricted epigenetic reprogramming in adulthood. Nat Commun. 2020 May 8;11(1):2316.

Li S, Ma F, Yokota T, et al. Metabolic reprogramming and epigenetic changes of vital organs in SARS-CoV-2 induced systemic toxicity. JCI Insight. 2020 Dec 7;145027.
Huang R, Zhou P. Double-edged effects of noncoding RNAs in responses to environmental genotoxic insults: Perspectives with regards to molecule-ecology network. Environ Pollut. 2019 Jan 9;247:64-71.
Tiffon C. The Impact of Nutrition and Environmental Epigenetics on Human Health and Disease. Int J Mol Sci. 2018 Nov 1;19(11).
Strakovsky RS, Schantz SL. Impacts of bisphenol A (BPA) and phthalate exposures on epigenetic outcomes in the human placenta. Environ Epigenet. 2018 Sep 7;4(3):dvy022.
He Y, Li Z. Epigenetic Environmental Memories in Plants: Establishment, Maintenance, and Reprogramming. Trends Genet. 2018 Nov;34(11):856-866.
Pucci M, Micioni Di Bonaventura MV, Wille-Bille A, et al. Environmental stressors and alcoholism development: Focus on molecular targets and their epigenetic regulation. Neurosci Biobehav Rev. 2018 Jul 11. doi: 10.1016/j.neubiorev.2018.07.004. [Epub ahead of print]
Friedrich T, Faivre L, Bäurle I, Schubert D. Chromatin-based mechanisms of temperature memory in plants. Plant Cell Environ. 2018 Jun 19. doi: 10.1111/pce.13373. [Epub ahead of print]
Smeester L, Fry RC. Long-Term Health Effects and Underlying Biological Mechanisms of Developmental Exposure to Arsenic. Curr Environ Health Rep. 2018 Mar;5(1):134-144.
Martin EM, Fry RC. Environmental Influences on the Epigenome: Exposure- Associated DNA Methylation in Human Populations. Annu Rev Public Health. 2018 Apr 1;39:309-333.
Mitra P, Sharma S, Purohit P, Sharma P. Clinical and molecular aspects of lead toxicity: An update. Crit Rev Clin Lab Sci. 2017 Nov - Dec;54(7-8):506-528.
Helsley RN, Zhou C. Epigenetic impact of endocrine disrupting chemicals on lipid homeostasis and atherosclerosis: a pregnane X receptor-centric view. Environ Epigenet. 2017 Oct 1;3(4).
Julian CG. Epigenomics and human adaptation to high altitude. J Appl Physiol (1985). 2017 Nov 1;123(5):1362-1370.
Brander SM, Biales AD, Connon RE. The Role of Epigenomics in Aquatic Toxicology. Environ Toxicol Chem. 2017 Oct;36(10):2565-2573.
Weinhouse C. Mitochondrial-epigenetic crosstalk in environmental toxicology. Toxicology. 2017 Nov 1;391:5-17.
Denhardt DT. Effect of stress on human biology: Epigenetics, adaptation, inheritance, and social significance. J Cell Physiol. 2018 Mar;233(3):1975-1984.
Dewan S, De Frenne P, Vanden Broeck A, Steenackers M, Vander Mijnsbrugge K, Verheyen K. Transgenerational effects in asexually reproduced offspring of Populus. PLoS One. 2018 Dec 6;13(12):e0208591.
Beauregard F, Angers B. Effect of a locally adapted genome on environmentally induced epigenetic variation. Environ Epigenet. 2018 Nov 26;4(4):dvy025.
Cook CJ, Wilson CC, Burness G. Impacts of environmental matching on the routine metabolic rate and mass of native and mixed-ancestry brook trout (Salvelinus fontinalis) fry. Conserv Physiol. 2018 May 8;6(1):coy023.
Eirin-Lopez JM, Putnam HM. Marine Environmental Epigenetics. Ann Rev Mar Sci. 2019 Jan 3;11:335-368.
Webster AP, Plant D, Ecker S, et al. Increased DNA methylation variability in rheumatoid arthritis-discordant monozygotic twins. Genome Med. 2018 Sep 4;10(1):64.
Tong C, Wen L, Xia Y, Leong P, et al. Protocol for a longitudinal twin birth cohort study to unravel the complex interplay between early-life environmental and genetic risk factors in health and

disease: the Chongqing Longitudinal Twin Study (LoTiS). BMJ Open. 2018 Feb 22;8(2):e017889.
Tozzi MG, Moscuzza F, Michelucci A, et al. ExtraUterine Growth Restriction (EUGR) in Preterm Infants: Growth Patterns, Nutrition, and Epigenetic Markers. A Pilot Study. Front Pediatr. 2018 Dec 20;6:408.
Strakovsky RS, Schantz SL. Impacts of bisphenol A (BPA) and phthalate exposures on epigenetic outcomes in the human placenta. Environ Epigenet. 2018 Sep 7;4(3):dvy022.
Sun W, Dong H, Becker AS, Dapito DH, et al. Cold-induced epigenetic programming of the sperm enhances brown adipose tissue activity in the offspring. Nat Med. 2018 Sep;24(9):1372-1383.
Padmanabhan N, Rakoczy J, Kondratowicz M, et al. Multigenerational analysis of sex-specific phenotypic differences at midgestation caused by abnormal folate metabolism. Environ Epigenet. 2017 Nov 3;3(4):dvx014.
Akemann C, Meyer DN, Gurdziel K, Baker TR. Developmental Dioxin Exposure Alters the Methylome of Adult Male Zebrafish Gonads. Front Genet. 2019 Jan 11;9:719.

Ashbrook DG, Hing B, Michalovicz LT, et al. Epigenetic impacts of stress priming of the neuroinflammatory response to sarin surrogate in mice: a model of Gulf War illness. J Neuroinflammation. 2018 17;15(1):86.
Cavalieri V, Spinelli G. Environmental epigenetics in zebrafish. Epigenetics Chromatin. 2017 5;10(1):46.
Xue J, Gharaibeh RZ, Pietryk EW, et al. Impact of vitamin D depletion during development on mouse sperm DNA methylation. Epigenetics. 2018;13(9):959-974.
Soubry A. POHaD: why we should study future fathers. Environ Epigenet. 2018 26;4(2):dvy007.
Barouki R, Melén E, Herceg Z, Beckers J, et al. Epigenetics as a mechanism linking developmental exposures to long-term toxicity. Environ Int. 2018 May;114:77-86.
Laubach ZM, Perng W, Dolinoy DC, Faulk CD, Holekamp KE, Getty T. Epigenetics and the maintenance of developmental plasticity: extending the signalling theory framework. Biol Rev Camb Philos Soc. 2018 Aug;93(3):1323-1338.
Van Baak TE, Coarfa C, Dugué PA, Fiorito G, et al. Epigenetic supersimilarity of monozygotic twin pairs. Genome Biol. 2018 Jan 9;19(1):2.
Hoffman DJ, Reynolds RM, Hardy DB. Developmental origins of health and disease: current knowledge and potential mechanisms. Nutr Rev. 2017 Dec 1;75(12):951-970.
Chang RC, Skiles WM, Chronister SS, et al. DNA methylation-independent growth restriction and altered developmental programming in a mouse model of preconception male alcohol exposure. Epigenetics. 2017;12(10):841-853.
Mochizuki K, Hariya N, Honma K, Goda T. Relationship between epigenetic regulation, dietary habits, and the developmental origins of health and disease theory. Congenit Anom (Kyoto). 2017 Nov;57(6):184-190.
St-Cyr S, McGowan PO. Adaptation or pathology? The role of prenatal stressor type and intensity in the developmental programing of adult phenotype. Neurotoxicol Teratol. 2018 Mar - Apr;66:113-124.
Lock MC, Botting KJ, Tellam RL, Brooks D, Morrison JL. Adverse Intrauterine Environment and Cardiac miRNA Expression. Int J Mol Sci. 2017 Dec 6;18(12).
Soubry A. Epigenetics as a Driver of Developmental Origins of Health and Disease: Did We Forget the Fathers? Bioessays. 2018 Jan;40(1).
Weber-Stadlbauer U. Epigenetic and transgenerational mechanisms in infection-mediated neurodevelopmental disorders. Transl Psychiatry. 2017 May 2;7(5):e1113.

Bale TL. Epigenetic and transgenerational reprogramming of brain development. Nat Rev Neurosci. 2015 Jun;16(6):332-44.
Nagy C, Turecki G. Transgenerational epigenetic inheritance: an open discussion. Epigenomics. 2015 Aug;7(5):781-90.
Wimmer ME, Vassoler FM, White SL, Schmidt HD, Sidoli S, Han Y, Garcia BA, Pierce RC. Impaired cocaine-induced behavioral plasticity in the male offspring of cocaine-experienced sires. Eur J Neurosci. 2018 Dec 19. doi: 10.1111/ejn.14310. [Epub ahead of print]
Sweatt JD. The Epigenetic Basis of Individuality. Curr Opin Behav Sci. 2019 Feb;25:51-56.
Dewan S, De Frenne P, Vanden Broeck A, Steenackers M, Vander Mijnsbrugge K, Verheyen K. Transgenerational effects in asexually reproduced offspring of Populus. PLoS One. 2018 Dec 6;13(12):e0208591.
Bughio F, Maggert KA. The peculiar genetics of the ribosomal DNA blurs the boundaries of transgenerational epigenetic inheritance. Chromosome Res. 2018 Dec 4. doi: 10.1007/s10577-018-9591-2. [Epub ahead of print]
Ben Maamar M, Nilsson E, Sadler-Riggleman I, Beck D, McCarrey JR, Skinner MK. Developmental origins of transgenerational sperm DNA methylation epimutations following ancestral DDT exposure. Dev Biol. 2019 Jan 15;445(2):280-293.
Jones BM, Robinson GE. Genetic accommodation and the role of ancestral plasticity in the evolution of insect eusociality. J Exp Biol. 2018 Nov 26;221(Pt 23). pii: jeb153163. doi: 10.1242/jeb.153163.
Woodhouse RM, Buchmann G, Hoe M, Harney DJ, Low JKK, Larance M, Boag PR, Ashe A. Chromatin Modifiers SET-25 and SET-32 Are Required for Establishment but Not Long-Term Maintenance of Transgenerational Epigenetic Inheritance. Cell Rep. 2018 Nov 20;25(8):2259-2272.e5
Chow YL, Sato F. Transgenerational lipid-reducing activity of benzylisoquinoline alkaloids in Caenorhabditis elegans. Genes Cells. 2019 Jan;24(1):70-81.
Skvortsova K, Iovino N, Bogdanović O. Functions and mechanisms of epigenetic inheritance in animals. Nat Rev Mol Cell Biol. 2018 Dec;19(12):774-790.
Liégard B, Baillet V, Etcheverry M, Joseph E, et al. Quantitative resistance to clubroot infection mediated by transgenerational epigenetic variation in Arabidopsis. New Phytol. 2018 Nov 4. doi: 10.1111/nph.15579. [Epub ahead of print]
Schmid MW, Heichinger C, Coman Schmid D, et al. Contribution of epigenetic variation to adaptation in Arabidopsis. Nat Commun. 2018 Oct 25;9(1):4446.
Klastrup LK, Bak ST, Nielsen AL. The influence of paternal diet on sncRNA-mediated epigenetic inheritance. Mol Genet Genomics. 2019 Feb;294(1):1-11.
Pfeiffer JR, Mutesa L, Uddin M. Traumatic Stress Epigenetics. Curr Behav Neurosci Rep. 2018 Mar;5(1):81-93.
Keinan-Boker L. Increased cancer incidence in Holocaust survivors and the implications for survivors of other extreme events. Expert Rev Anticancer Ther. 2018 Nov;18(11):1059-1062.
Bošković A, Rando OJ. Transgenerational Epigenetic Inheritance. Annu Rev Genet. 2018 Nov 23;52:21-41.
Paiva JT, De Resende MDV, Resende RT, et al. A note on transgenerational epigenetics affecting egg quality traits in meat-type quail. Br Poult Sci. 2018 Sep 21:1-5.
Meloni M, Müller R. Transgenerational epigenetic inheritance and social responsibility: perspectives from the social sciences. Environ Epigenet. 2018 Jul 31;4(2):dvy019.
Nilsson EE, Sadler-Riggleman I, Skinner MK. Environmentally induced epigenetic transgenerational inheritance of disease. Environ Epigenet. 2018 Jul 17;4(2):dvy016.

Hu L, Xiao P, Jiang Y, Dong M, Chen Z, Li H, Hu Z, Lei A, Wang J. Transgenerational Epigenetic Inheritance Under Environmental Stress by Genome-Wide DNA Methylation Profiling in Cyanobacterium. Front Microbiol. 2018 Jul 4;9:1479.
Goldberg LR, Gould TJ. Multigenerational and transgenerational effects of paternal exposure to drugs of abuse on behavioral and neural function. Eur J Neurosci. 2018 Jun 27. doi: 10.1111/ejn.14060. [Epub ahead of print]
Yu R, Wang X, Moazed D. Epigenetic inheritance mediated by coupling of RNAi and histone H3K9 methylation. Nature. 2018 Jun;558(7711):615-619.
Paiva JT, de Resende MDV, Resende RT, de Oliveira HR, Silva HT, Caetano GC, Lopes PS, Silva FF. Transgenerational epigenetic variance for body weight in meat quails. J Anim Breed Genet. 2018 Jun;135(3):178-185.
Champroux A, Cocquet J, Henry-Berger J, Drevet JR, Kocer A. A Decade of Exploring the Mammalian Sperm Epigenome: Paternal Epigenetic and Transgenerational Inheritance. Front Cell Dev Biol. 2018 May 15;6:50.
Youssef NA, Lockwood L, Su S, Hao G, Rutten BPF. The Effects of Trauma, with or without PTSD, on the Transgenerational DNA Methylation Alterations in Human Offsprings. Brain Sci. 2018 May 8;8(5). pii: E83. doi: 10.3390/brainsci8050083.
Ben Maamar M, Sadler-Riggleman I, Beck D, McBirney M, Nilsson E, Klukovich R, Xie Y, Tang C, Yan W, Skinner MK. Alterations in sperm DNA methylation, non-coding RNA expression, and histone retention mediate vinclozolin-induced epigenetic transgenerational inheritance of disease. Environ Epigenet. 2018 Apr 26;4(2):dvy010.
Guerrero-Bosagna C, Morisson M, Liaubet L, Rodenburg TB, de Haas EN, Košťál Ľ, Pitel F. Transgenerational epigenetic inheritance in birds. Environ Epigenet. 2018 26;4(2):dvy008.
Soubry A. POHaD: why we should study future fathers. Environ Epigenet. 2018 26;4(2):dvy007.
Herrera CM, Alonso C, Medrano M, Pérez R, Bazaga P. Transgenerational epigenetics: Inheritance of global cytosine methylation and methylation-related epigenetic markers in the shrub Lavandula latifolia. Am J Bot. 2018 Apr;105(4):741-748.
Skinner MK, Ben Maamar M, Sadler-Riggleman I, Beck D, Nilsson E, McBirney M, Klukovich R, Xie Y, Tang C, Yan W. Alterations in sperm DNA methylation, non-coding RNA and histone retention associate with DDT-induced epigenetic transgenerational inheritance of disease. Epigenetics Chromatin. 2018 Feb 27;11(1):8.
Kawai K, Harada T, Ishikawa T, Sugiyama R, Kawamura T, Yoshida A, Tsutsumi O, Ishino F, Kubota T, Kohda T. Parental age and gene expression profiles in individual human blastocysts. Sci Rep. 2018 Feb 5;8(1):2380.
Pearson BL, Ehninger D. Impact of paternal nutrition on epigenetic patterns. Epigenomics. 2018 Feb;10(2):115-117.
Williams BP, Gehring M. Stable transgenerational epigenetic inheritance requires a DNA methylation-sensing circuit. Nat Commun. 2017 Dec 14;8(1):2124.
Ruden DM, Gurdziel K, Aschner M. Frontiers in Toxicogenomics in the Twenty-First Century-the Grand Challenge: To Understand How the Genome and Epigenome Interact with the Toxic Environment at the Single-Cell, Whole-Organism, and Multi-Generational Level. Front Genet. 2017 Nov 9;8:173.
Annacondia ML, Magerøy MH, Martinez G. Stress response regulation by epigenetic mechanisms: changing of the guards. Physiol Plant. 2018 Feb;162(2):239-250.
Moisiadis VG, Constantinof A, Kostaki A, Szyf M, Matthews SG. Prenatal Glucocorticoid Exposure Modifies Endocrine Function and Behaviour for 3 Generations Following Maternal and Paternal Transmission. Sci Rep. 2017 Sep 18;7(1):11814.

Vaiserman AM, Koliada AK, Jirtle RL. Non-genomic transmission of longevity between generations: potential mechanisms and evidence across species. Epigenetics Chromatin. 2017 27;10(1):38.
Pilling OA, Rogers AJ, Gulla-Devaney B, Katz LA. Insights into transgenerational epigenetics from studies of ciliates. Eur J Protistol. 2017 Oct;61(Pt B):366-375.
Morgado L, Preite V, Oplaat C, Anava S, Ferreira de Carvalho J, Rechavi O, Johannes F, Verhoeven KJF. Small RNAs Reflect Grandparental Environments in Apomictic Dandelion. Mol Biol Evol. 2017 Aug 1;34(8):2035-2040.
Aliaga B, Bulla I, Mouahid G, Duval D, Grunau C. Universality of the DNA methylation codes in Eucaryotes. Sci Rep. 2019 Jan 17;9(1):173.
Daniūnaitė K, Jarmalaitė S, Kriukienė E. Epigenomic technologies for deciphering circulating tumor DNA. Curr Opin Biotechnol. 2019 Feb;55:23-29.
Wang L, Marek GW 3rd, Hlady RA, Wagner RT, Zhao X, Clark VC, Fan AX, Liu C, Brantly M, Robertson KD. Alpha-1 antitrypsin deficiency liver disease, mutational homogeneity modulated by epigenetic heterogeneity with links to obesity. Hepatology. 2019 Jan 25. doi: 10.1002/hep.30526. [Epub ahead of print]
Kallimasioti-Pazi EM, Thelakkad Chathoth K, Taylor GC, Meynert A, Ballinger T, Kelder MJE, Lalevée S, Sanli I, Feil R, Wood AJ. Heterochromatin delays CRISPR-Cas9 mutagenesis but does not influence the outcome of mutagenic DNA repair. PLoS Biol. 2018 Dec 12;16(12):e2005595.
Khamlichi AA, Feil R. Parallels between Mammalian Mechanisms of Monoallelic Gene Expression. Trends Genet. 2018 Dec;34(12):954-971.
Horton JR, Woodcock CB, Chen Q, Liu X, et al. Structure-Based Engineering of Irreversible Inhibitors against Histone Lysine Demethylase KDM5A. J Med Chem. 2018 Nov 15. doi: 10.1021/acs.jmedchem.8b01219. [Epub ahead of print]
Wilkinson AW, Diep J, Dai S, Liu S, Ooi YS, et al. SETD3 is an actin histidine methyltransferase that prevents primary dystocia. Nature. 2019 Jan;565(7739):372-376.
Ehrlich KC, Lacey M, Ehrlich M. Tissue-specific epigenetics of atherosclerosis-related ANGPT and ANGPTL genes. Epigenomics. 2019 Feb;11(2):169-186.
Peixoto, Renaude, Boyer-Guittaut, Hervouet. Epigenetics, a key player of immunotherapy resistance. Cancer Drug Resist 2018;1:219-29.
Sierra MI, Valdés A, Fernández AF, Torrecillas R, Fraga MF. The effect of exposure to nanoparticles and nanomaterials on the mammalian epigenome. Int J Nanomedicine. 2016;11:6297-6306.
Loche E, Ozanne SE. Early nutrition, epigenetics, and cardiovascular disease. Curr Opin Lipidol. 2016 Oct;27(5):449-58.
Derghal A, Djelloul M, Trouslard J, Mounien L. An Emerging Role of micro-RNA in the Effect of the Endocrine Disruptors. Front Neurosci. 2016 Jun 30;10:318.
Lambertini L, Byun HM. Mitochondrial Epigenetics and Environmental Exposure. Curr Environ Health Rep. 2016 Sep;3(3):214-24.
Karlsson O, Baccarelli AA. Environmental Health and Long Non-coding RNAs. Curr Environ Health Rep. 2016 Sep;3(3):178-87.
Larriba E, del Mazo J. Role of Non-Coding RNAs in the Transgenerational Epigenetic Transmission of the Effects of Reprotoxicants. Int J Mol Sci. 2016 Mar 25;17(4):452.
Bhattacharjee P, Paul S, Bhattacharjee P. Risk of occupational exposure to asbestos, silicon and arsenic on pulmonary disorders: Understanding the genetic-epigenetic interplay and future prospects. Environ Res. 2016 May;147:425-34.
Schmidt E, Kornfeld JW. Decoding Lamarck-transgenerational control of metabolism by noncoding RNAs. Pflugers Arch. 2016 Jun;468(6):959-69.

Prokopuk L, Western PS, Stringer JM. Transgenerational epigenetic inheritance: adaptation through the germline epigenome? Epigenomics. 2015 Aug;7(5):829-46.
Kochmanski J, Montrose L, Goodrich JM, Dolinoy DC. Environmental Deflection: The Impact of Toxicant Exposures on the Aging Epigenome. Toxicol Sci. 2017 Jan 13. pii: kfx005. [Epub ahead of print]
Segal NL, Montoya YS, Loke YJ, Craig JM. Identical twins doubly exchanged at birth: a case report of genetic and environmental influences on the adult epigenome. Epigenomics. 2017 9(1):5-12.
Shojaei Saadi HA, Fournier É, Vigneault C, Blondin P, Bailey J, Robert C. Genome-wide analysis of sperm DNA methylation from monozygotic twin bulls. Reprod Fertil Dev. 2016 Jan 12. doi: 10.1071/RD15384. [Epub ahead of print]
Yet I, Tsai PC, Castillo-Fernandez JE, Carnero-Montoro E, Bell JT. Genetic and environmental impacts on DNA methylation levels in twins. Epigenomics. 2016 Jan;8(1):105-17.
Sharma A. Transgenerational epigenetics: Integrating soma to germline communication with gametic inheritance. Mech Ageing Dev. 2017 Jan 16. pii: S0047-6374(16)30248-2. [Epub ahead of print]
Ho SM, Cheong A, Adgent MA, et al. Environmental factors, epigenetics, and developmental origin of reproductive disorders. Reprod Toxicol. 2016 Jul 12. pii: S0890-6238(16)30268-4. doi: 10.1016/j.reprotox.2016.07.011. [Epub ahead of print]
Hanson MA, Skinner MK. Developmental origins of epigenetic transgenerational inheritance. Environ Epigenet. 2016;2(1). pii: dvw002. Epub 2016 Mar 6.
Guillette LJ Jr, Parrott BB, Nilsson E, Haque MM, Skinner MK. Epigenetic programming alterations in alligators from environmentally contaminated lakes. Gen Comp Endocrinol. 2016 Nov 1;238:4-12.
Veazey KJ, Parnell SE, Miranda RC, Golding MC. Dose-dependent alcohol-induced alterations in chromatin structure persist beyond the window of exposure and correlate with fetal alcohol syndrome birth defects. Epigenetics Chromatin. 2015 Sep 28;8:39.
Xin F, Susiarjo M, Bartolomei MS. Multigenerational and transgenerational effects of endocrine disrupting chemicals: A role for altered epigenetic regulation? Semin Cell Dev Biol. 2015 Jul;43:66-75.
Rodgers AB, Bale TL. Germ Cell Origins of Posttraumatic Stress Disorder Risk: The Transgenerational Impact of Parental Stress Experience. Biol Psychiatry. 2015 Sep 1;78(5):307-14.
Hernández-Aguilera A, Fernández-Arroyo S, Cuyàs E, et al. Epigenetics and nutrition-related epidemics of metabolic diseases: Current perspectives and challenges. Food Chem Toxicol. 2016 Oct;96:191-204.
Loche E, Ozanne SE. Early nutrition, epigenetics, and cardiovascular disease. Curr Opin Lipidol. 2016 Oct;27(5):449-58.
Joubert BR, Felix JF, Yousefi P, et al. DNA Methylation in Newborns and Maternal Smoking in Pregnancy: Genome-wide Consortium Meta-analysis. Am J Hum Genet. 2016 Apr 7;98(4):680-96.
Lahiri DK, Maloney B, Bayon BL, Chopra N, White FA, Greig NH, Nurnberger JI. Transgenerational latent early-life associated regulation unites environment and genetics across generations. Epigenomics. 2016 Mar;8(3):373-87.
Joubert BR, den Dekker HT, Felix JF, et al. Maternal plasma folate impacts differential DNA methylation in an epigenome-wide meta-analysis of newborns. Nat Commun. 2016 Feb 10;7:10577.

Marsit CJ. Placental Epigenetics in Children's Environmental Health. Semin Reprod Med. 2016 Jan;34(1):36-41.
Vargas AO, Krabichler Q, Guerrero-Bosagna C. An Epigenetic Perspective on the Midwife Toad Experiments of Paul Kammerer (1880-1926). J Exp Zool B Mol Dev Evol. 2017 Jan;328(1-2):179-192.
Shama LN, Mark FC, Strobel A, et al. Transgenerational effects persist down the maternal line in marine sticklebacks: gene expression matches physiology in a warming ocean. Evol Appl. 2016 Feb 28;9(9):1096-1111.
Herman JJ, Sultan SE. DNA methylation mediates genetic variation for adaptive transgenerational plasticity. Proc Biol Sci. 2016 Sep 14;283(1838).
Schagdarsurengin U, Steger K. Epigenetics in male reproduction: effect of paternal diet on sperm quality and offspring health. Nat Rev Urol. 2016 Oct;13(10):584-95.
Bruner-Tran KL, Gnecco J, Ding T, Glore DR, Pensabene V, Osteen KG. Exposure to the environmental endocrine disruptor TCDD and human reproductive dysfunction: Translating lessons from murine models. Reprod Toxicol. 2016 Jul 14. pii: S0890-6238(16)30264-7. doi: 10.1016/j.reprotox.2016.07.007. [Epub ahead of print]
Schuster A, Skinner MK, Yan W. Ancestral vinclozolin exposure alters the epigenetic transgenerational inheritance of sperm small noncoding RNAs. Environ Epigenet. 2016;2(1). pii: dvw001. Epub 2016 Mar 9.
Haque MM, Nilsson EE, Holder LB, Skinner MK. Genomic Clustering of differential DNA methylated regions (epimutations) associated with the epigenetic transgenerational inheritance of disease and phenotypic variation. BMC Genomics. 2016 Jun 1;17:418.
van Otterdijk SD, Michels KB. Transgenerational epigenetic inheritance in mammals: how good is the evidence? FASEB J. 2016 Jul;30(7):2457-65.
Larriba E, del Mazo J. Role of Non-Coding RNAs in the Transgenerational Epigenetic Transmission of the Effects of Reprotoxicants. Int J Mol Sci. 2016 Mar 25;17(4):452.
de Castro Barbosa T, Ingerslev LR, Alm PS, et al. High-fat diet reprograms the epigenome of rat spermatozoa and transgenerationally affects metabolism of the offspring. Mol Metab. 2015 Dec 25;5(3):184-97.
Lahiri DK, Maloney B, Bayon BL, et al. Transgenerational latent early-life associated regulation unites environment and genetics across generations. Epigenomics. 2016 Mar;8(3):373-87.
Junien C, Panchenko P, Fneich S, et al. [Epigenetics in transgenerational responses to environmental impacts: from facts and gaps]. [Article in French] Med Sci (Paris). 2016 Jan;32(1):35-44.
Walsh MR, Castoe T, Holmes J, et al. Local adaptation in transgenerational responses to predators. Proc Biol Sci. 2016 Jan 27;283(1823).
Stuppia L, Franzago M, Ballerini P, Gatta V, Antonucci I. Epigenetics and male reproduction: the consequences of paternal lifestyle on fertility, embryo development, and children lifetime health. Clin Epigenetics. 2015 Nov 11;7:120.
Nilsson EE, Skinner MK. Environmentally Induced Epigenetic Transgenerational Inheritance of Reproductive Disease. Biol Reprod. 2015 Dec;93(6):145.
Stel J, Legler J. The Role of Epigenetics in the Latent Effects of Early Life Exposure to Obesogenic Endocrine Disrupting Chemicals. Endocrinology. 2015 Oct;156(10):3466-72.
Clarke HJ, Vieux KF. Epigenetic inheritance through the female germ-line: The known, the unknown, and the possible. Semin Cell Dev Biol. 2015 Jul;43:106-16.
Barua S, Junaid MA. Lifestyle, pregnancy and epigenetic effects. Epigenomics. 2015 Feb;7(1):85-102.

Tan Q, Christiansen L, von Bornemann Hjelmborg J, Christensen K. Twin methodology in epigenetic studies. J Exp Biol. 2015 Jan 1;218(Pt 1):134-139.
Bonasio R. The expanding epigenetic landscape of non-model organisms. J Exp Biol. 2015 Jan 1;218(Pt 1):114-122.
Castillo-Fernandez JE, Spector TD, Bell JT. Epigenetics of discordant monozygotic twins: implications for disease. Genome Med. 2014 Jul 31;6(7):60.
Giuliani C, Bacalini MG, Sazzini M, et al. The epigenetic side of human adaptation: hypotheses, evidences and theories. Ann Hum Biol. 2015 Jan;42(1):1-9.
Goddard ME, Whitelaw E. The use of epigenetic phenomena for the improvement of sheep and cattle. Front Genet. 2014 Aug 21;5:247.
Crews D, Gillette R, Miller-Crews I, Gore AC, Skinner M. Nature, nurture and epigenetics. Mol Cell Endocrinol. 2014 Dec;398(1-2):42-52.
Sales NM, Pelegrini PB, Goersch MC. Nutrigenomics: definitions and advances of this new science. J Nutr Metab. 2014;2014:202759.
Burggren WW, Crews D. Epigenetics in comparative biology: why we should pay attention. Integr Comp Biol. 2014 Jul;54(1):7-20.
Horowitz M. Heat acclimation, epigenetics, and cytoprotection memory. Compr Physiol. 2014 Jan;4(1):199-230.
Orozco-Solis R, Sassone-Corsi P. Epigenetic control and the circadian clock: linking metabolism to neuronal responses. Neuroscience. 2014 Apr 4;264:76-87.
Bakulski KM, Fallin MD. Epigenetic epidemiology: promises for public health research. Environ Mol Mutagen. 2014 Apr;55(3):171-83.
Byun HM, Baccarelli AA. Environmental exposure and mitochondrial epigenetics: study design and analytical challenges. Hum Genet. 2014 Mar;133(3):247-57.
Zannas AS, West AE. Epigenetics and the regulation of stress vulnerability and resilience. Neuroscience. 2014 Apr 4;264:157-70.
Damaschke NA, Yang B, Bhusari S, Svaren JP, Jarrard DF. Epigenetic susceptibility factors for prostate cancer with aging. Prostate. 2013 Dec;73(16):1721-30.
Lock M. The epigenome and nature/nurture reunification: a challenge for anthropology. Med Anthropol. 2013;32(4):291-308.
Giordani L, Puri PL. Epigenetic control of skeletal muscle regeneration: Integrating genetic determinants and environmental changes. FEBS J. 2013 Sep;280(17):4014-25.
DelCurto H, Wu G, Satterfield MC. Nutrition and reproduction: links to epigenetics and metabolic syndrome in offspring. Curr Opin Clin Nutr Metab Care. 2013 Jul;16(4):385-91.
Collotta M, Bertazzi PA, Bollati V. Epigenetics and pesticides. Toxicology. 2013 May 10;307:35-41.
Jensen P. Transgenerational epigenetic effects on animal behaviour. Prog Biophys Mol Biol. 2013 Dec;113(3):447-54.
Sookoian S, Gianotti TF, Burgueño AL, Pirola CJ. Fetal metabolic programming and epigenetic modifications: a systems biology approach. Pediatr Res. 2013 Apr;73(4 Pt 2):531-42.
Guerrero-Bosagna CM, Skinner MK. Environmental epigenetics and phytoestrogen/phytochemical exposures. J Steroid Biochem Mol Biol. 2014 Jan;139:270-6.
McEwen BS, Getz L. Lifetime experiences, the brain and personalized medicine: an integrative perspective. Metabolism. 2013 Jan;62 Suppl 1:S20-6.
Huidobro C, Fernandez AF, Fraga MF. Aging epigenetics: causes and consequences. Mol Aspects Med. 2013 Jul-Aug;34(4):765-81.
Marsit CJ. Influence of environmental exposure on human epigenetic regulation. J Exp Biol. 2015 Jan 1;218(Pt 1):71-79.

Kamstra JH, Aleström P, Kooter JM, Legler J. Zebrafish as a model to study the role of DNA methylation in environmental toxicology. Environ Sci Pollut Res Int. 2014 Aug 31. [Epub ahead of print]

Ray PD, Yosim A, Fry RC. Incorporating epigenetic data into the risk assessment process for the toxic metals arsenic, cadmium, chromium, lead, and mercury: strategies and challenges. Front Genet. 2014 Jul 16;5:201.

Williams TD, Mirbahai L, Chipman JK. The toxicological application of transcriptomics and epigenomics in zebrafish and other teleosts. Brief Funct Genomics. 2014 Mar;13(2):157-71.

Paoloni-Giacobino A. Epigenetic effects of methoxychlor and vinclozolin on male gametes. Vitam Horm. 2014;94:211-27.

ENVIRONMENTAL EPIGENETICS

Environmental Epigenetics, 2022, **8(1)**, 1–9
DOI: https://doi.org/10.1093/eep/dvac001
Review Article

Role of epigenetic transgenerational inheritance in generational toxicology

Eric E. Nilsson, Millissia Ben Maamar and Michael K. Skinner*

Center for Reproductive Biology, School of Biological Sciences, Washington State University, Pullman, WA 99164-4236, USA
*Correspondence address. Center for Reproductive Biology, School of Biological Sciences, Washington State University, Pullman, WA 99164-4236, USA.
Tel: +509-335-1524; E-mail: skinner@wsu.edu

Abstract

Many environmental toxicants have been shown to be associated with the transgenerational inheritance of increased disease susceptibility. This review describes the generational toxicity of some of these chemicals and their role in the induction of epigenetic transgenerational inheritance of disease. Epigenetic factors include DNA methylation, histone modifications, retention of histones in sperm, changes to chromatin structure, and expression of non-coding RNAs. For toxicant-induced epigenetic transgenerational inheritance to occur, exposure to a toxicant must result in epigenetic changes to germ cells (sperm or eggs) since it is the germ cells that carry molecular information to subsequent generations. In addition, the epigenetic changes induced in transgenerational generation animals must cause alterations in gene expression in these animals' somatic cells. In some cases of generational toxicology, negligible changes are seen in the directly exposed generations, but increased disease rates are seen in transgenerational descendants. Governmental policies regulating toxicant exposure should take generational effects into account. A new approach that takes into consideration generational toxicity will be needed to protect our future populations.

Key words: epigenetics; generational toxicology; transgenerational

Introduction

Previous studies have demonstrated the ability of environmental toxicants to promote the epigenetic transgenerational inheritance of disease, which can be termed "generational toxicology." Therefore, exposure to environmental toxicants can increase disease rates in subsequent generations not directly exposed [1]. Although the field of toxicology has focused on direct exposure toxicity, generational impacts have not been previously considered due in part to the lack of continued direct exposure. This review describes the molecular processes and factors that affect the epigenetic transgenerational inheritance of disease related to ancestral chemical toxicant exposure.

The term epigenetics was originally coined by C. H. Waddington in the 1940s to refer to how an organism's genes and its environment can interact to result in non-Mendelian inheritance of phenotypes [2, 3]. In more current usage, epigenetics is defined as "the molecular factors and processes around the DNA that regulate genome activity independent of DNA sequence, and are mitotically stable" [4]. Epigenetic molecular factors include DNA methylation [5, 6], histone modifications [7], changes to chromatin structure [8], expression of non-coding RNAs (ncRNAs) [9, 10], and RNA methylation [11]. These epigenetic factors and their interactions together comprise what is termed the epigenome. Changes to epigenetic factors are a critical mechanism by which organisms respond to their environment, altering somatic cell gene expression to change physiology [12]. In addition, epigenetic changes underlie the differentiation of stem cells into the many differentiated cell types in an organism [4, 13, 14]. Therefore, cellular differentiation and cell specificity is, in large part, determined by epigenetics. Epigenetic mechanisms are a critical part of all normal biological processes, including how the environment influences biology.

Molecular Epigenetic Mechanisms

There are several epigenetic factors that act around the DNA to regulate gene expression in cells. The most studied epigenetic factor is DNA methylation. This involves the chemical addition of functional methyl groups to DNA. DNA methylation occurs primarily at cytosine bases that are adjacent to guanine, termed CpG residues, to form 5-methylcytosine (5mC) [15]. Other chemical modifications of CpG residues can also occur. The Ten-Eleven Translocation (TET) enzyme family can successively oxidize 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine and 5-carboxylcytosine [16]. Typically, 5mC is thought to repress transcription, while 5hmC is thought to be permissive of transcription [17, 18]. Another important function of TET family enzymes is to remove DNA methylation during early embryonic development and cellular differentiation to help form embryonic stem cells [19–21]. DNA methylation can also occur at adenosine residues to form N(6)-methyladenine (N6-mA)

Received 15 September 2021; **revised** 4 November 2021; **accepted** 3 February 2022

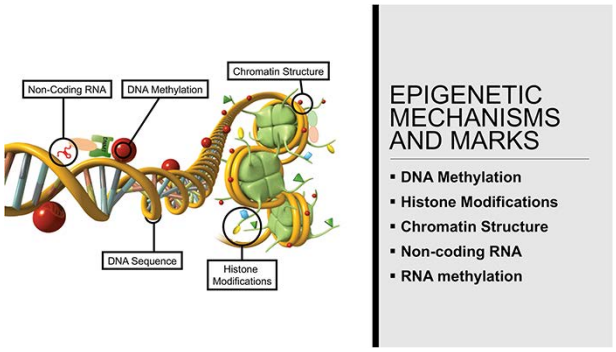


Figure 1: Schematic representation of the primary epigenetic factors and processes of non-coding RNA, DNA methylation, chromatin structure, histone modifications, and DNA structure presented. Modified from Nilsson *et al.* [1]

[22]. N(6)-mA, once thought to only occur in prokaryotic organisms, has been described to occur in mammalian embryonic stem cells. DNA methylation has a critical role in regulating gene expression and chromatin structure, which is present in all cells and organisms (Fig. 1). The optimal DNA methylation procedures use genome-wide analyses, such as methylated DNA immunoprecipitation (MeDIP) and bisulfite sequencing, compared to array technology, which assesses a few percent of the genome [23].

DNA is wrapped around histone proteins to form nucleosomes. Another epigenetic factor involves the chemical modification of nucleosome histones that act to regulate gene expression [24, 25]. These histone modifications include lysine acetylation, lysine and arginine methylation, arginine citrullination, lysine ubiquitination, lysine sumoylation, ADP-ribosylation, proline isomerization, and serine/threonine/tyrosine phosphorylation [24]. The effects of these modifications include changing chromatin structure, suppressing gene expression in areas of heterochromatin, and recruiting transcriptional cofactors [25, 26]. Additional histone-related epigenetic factors include the use of histone variants, changes to the spacing between nucleosomes, and the positioning of chromatin within the nucleus [26]. These factors act together to regulate gene expression by controlling gene accessibility and recruitment of transcriptional cofactors [27, 28], (Fig. 1). The optimal genome-wide histone modification technology uses chromatin immunoprecipitation procedures [29]. ncRNA molecules can act as epigenetic factors [30, 31]. These are RNA sequences that do not rely on complimentary base sequences to bind and act to regulate gene expression [32]. ncRNAs have been shown to regulate embryogenesis and other developmental processes [33]. Long ncRNAs [30] and small ncRNAs regulate gene expression through DNA and protein binding to alter gene expression and are present in all cell types and organisms [30], (Fig. 1). An example includes transfer RNA-derived small tRNA fragments [34] that can influence gene expression and are present in sperm and can act on subsequent generations to alter phenotype [35, 36]. The optimal genome-wide technology used for ncRNA involves direct RNA sequencing [37].

Methylation of RNA can affect gene expression and so is considered another epigenetic factor [38]. Methylation of adenosine to form N6-mA is the most common epigenetic modification of the internal RNA sequence. This is a reversible modification and is associated with post-transcriptional regulation [39, 40]. Another modification of RNA that can occur is methylation of cytosine (m3C) in both mRNA and tRNA [41]. These epigenetic modifications of RNA all regulate RNA structure and gene expression (Fig. 1). The optimal genome-wide analysis of RNA methylation uses immunoprecipitation and RNA sequencing [42].

The three-dimensional coiling and looping of DNA and its associated proteins within the nucleus is termed chromatin structure and is itself an epigenetic factor [8]. The structure of chromatin affects the accessibility of genes to transcriptional machinery and can be affected by several of the other epigenetic factors, (Fig. 1). The best example is the compacted chromatin structure of heterochromatin that represses gene expression and that is promoted by hypermethylation of DNA versus the less compacted euchromatin that is associated with active gene expression and hypomethylation of DNA [24]. The optimal genome-wide technology for chromatin structure analysis also uses chromatin immunoprecipitation procedures [29].

Epigenetic Transgenerational Inheritance

Epigenetic information can be passed from one generation to another through sperm or eggs. If an organism is exposed to an environmental factor, such as a toxicant, epigenetic changes can be induced both in the somatic cells of the individual exposed, as well as in the directly exposed germ cells of the organism (Fig. 2). When epigenetic changes due to direct exposure of germ cells are passed on to affect the subsequent generation, this is termed multigenerational epigenetic inheritance [43]. In mammals, multigenerational inheritance can occur when males or females of a founder F0 generation are exposed to an environmental factor, and their epigenetically altered germ cells go on to form the F1 generation (Fig. 3). When gestating, F0-generation females are exposed to an environmental factor, then their oocytes, and the germ cells of each developing fetus, are also directly exposed. Therefore, the F2 generation descendants of exposed pregnant females are still considered to be the result of multigenerational epigenetic inheritance (Fig. 3).

Epigenetic transgenerational inheritance is defined as "germline-mediated inheritance of epigenetic information between generations in the absence of continued direct environmental influences that leads to phenotypic variation" [4]. If males or non-pregnant females of the F0 generation are exposed to an environmental factor, then epigenetic changes seen in the unexposed F2 generation grand-offspring are an example of epigenetic transgenerational inheritance (Fig. 3). Similarly, if pregnant females are exposed, then the F3 generation great-grand-offspring are the first generation that can exhibit epigenetic transgenerational inheritance [43].

The Agouti mouse model is a well-studied example of epigenetic multigenerational inheritance. Pregnant Agouti mice that are fed a diet rich in methyl donors show increased methylation of a methylation-sensitive allele of the Agouti gene, leading to a coat color change in their F1 generation offspring [44]. This coat color change is not passed on to the F2 or the transgenerational F3 generation. Rather, the normal process of demethylation and remethylation that occurs during germline development resets the methylation state of the Agouti allele to its original level, and a more normal coat color occurs [45].

Examples of transgenerational inheritance are well established in the literature (reviewed in [1]). Early studies were performed by Conrad Waddington in the 1940s, who coined the term "epigenetic" [46]. In these studies, fruit flies (*Drosophila melanogaster*) were exposed to a heat shock that induced changes in wing structure that persisted for more than 16 generations. One of the first

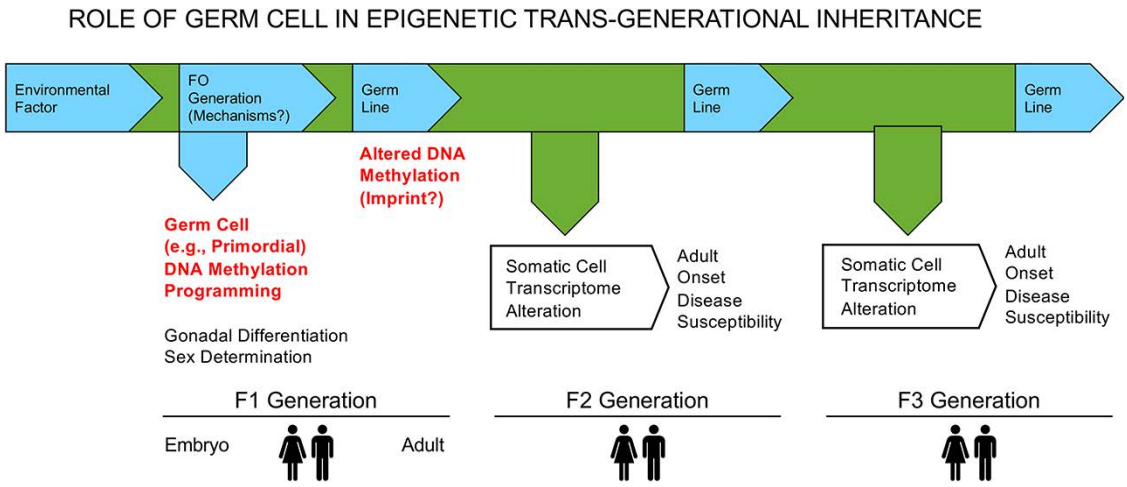


Figure 2: Role of germ cell in epigenetic transgenerational inheritance. The exposure of an F0 generation gestating female promotes an epigenetic alteration in the germ cell programming of the F1 generation fetus. The F1 generation adult passes the germ cell epimutations to the zygote and early embryo to alter the embryonic stem cell epigenetics and transcriptome to impact all developing somatic cell epigenetics and transcriptomes to promote cell and tissue disease susceptibility. The altered germ cell epigenetics is then transgenerationally transmitted to subsequent generations. Modified from Nilsson *et al.* [1]

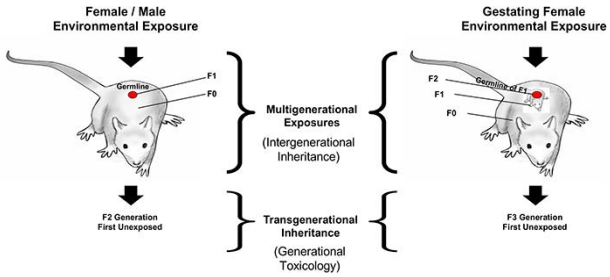


Figure 3: Environmentally induced transgenerational epigenetic inheritance: schematic of environmental exposure and affected generations for both gestating female and adult male or female. The multigenerational direct exposures are indicated in contrast to the transgenerational generation having no direct exposure. Modified from Nilsson *et al.* [1]

studies in mammals to document molecular epigenetic changes that were associated with the transgenerational inheritance of disease involved exposing pregnant rats to the agricultural fungicide and anti-androgenic endocrine disruptor vinclozolin [47]. The F3 generation descendants of the exposed pregnant rats had increased rates of reproductive abnormalities such as testicular germ cell apoptosis and decreased sperm motility. This was associated with altered DNA methylation in the F3 generation sperm. Subsequent studies showed that vinclozolin exposure resulted in the transgenerational inheritance of increased susceptibility to testis, prostate, and kidney disease, pubertal onset abnormalities, ovarian disease, mammary tumors, and an increased obesity rate in females [48–51]. Subsequently, many environmental toxicants have been shown to be associated with the transgenerational inheritance of increased disease susceptibility (Table 1). These environmental toxicants have been shown to impact a variety of different species from plants to humans (Fig. 4). This review will focus on the generational toxicity of these substances and their role in epigenetic transgenerational inheritance of disease.

Phthalates are plastics-derived endocrine disrupting compounds that have been shown to induce transgenerational effects in mice (Table 1). These effects include changes to male behaviors and to female corticosterone levels [52] and alterations in ovarian folliculogenesis and progesterone levels in females [53]. Exposure of mice to the plastics-derived compound bisphenol A (BPA) induced transgenerational changes in social behavior and in the expression of brain hormones, such as vasopressin and oxytocin [54]. Ancestral exposure to BPA also effects imprinted gene methylation and gene expression in the brains of mice [55]. Exposure of zebrafish to BPA results in a transgenerational increase in heart disorders [56]. Medaka fish ancestrally exposed to BPA or ethinylestradiol, an estrogenic environmental toxicant from birth control pills, show transgenerational reductions in fertility [57]. Exposure of pregnant rats to a mixture of BPA and phthalates was shown to increase the incidence of pubertal

Table 1: Environmental toxicant induction of epigenetic transgenerational inheritance: generational toxicology

Toxicants	References
Vinclozolin	[47–51, 84, 85, 92, 95, 98, 99, 101, 103, 104]
TCDD/dioxin	[68]
Plastics compounds (BPA, phthalates DEHP and DBP)	[52–59]
Jet fuel (JP8) (hydrocarbon mixture)	[62]
Pesticides and insect repellent (permethrin and DEET)	[67]
DDT	[61, 87, 92, 96, 104]
Methoxychlor	[66]
Chlordecone	[102]
Methylmercury	[76]
Lead	[105]
Arsenic	[63, 70–74]
Atrazine	[64, 65]
Glyphosate	[86, 93]
Decabromodiphenyl ether (BDE-209)	[88]
Tributyltin	[60]
5-azacytidine	[77]
Ethanol	[75]
Benzo[a]pyrene	[69]
Genistein	[79]

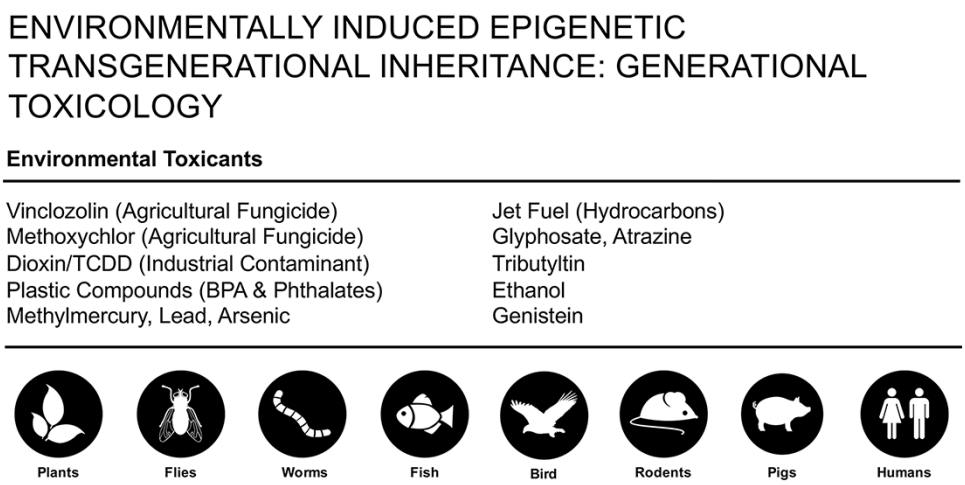


Figure 4: Environmentally induced epigenetic transgenerational inheritance. Various exposures and species investigated

abnormalities, testis disease, and ovarian disease in the transgenerational F3 generation [58]. In the nematode worm *C. elegans*, exposure to nanoplastic particles resulted in a transgenerational decline in reproduction [59].

Tributyltin is an environmental toxicant and endocrine disruptor with obesogenic properties that has been shown to induce the transgenerational inheritance of obesity and hepatic steatosis in mice [60]. Other toxicants known to induce epigenetic transgenerational inheritance of obesity in rats include dichlorodiphenyltrichloroethane (DDT) [61], a mixture of BPA and phthalates [58], and jet fuel hydrocarbons [62]. In mice, exposure to arsenic was shown to transgenerationally increase adiposity in males [63].

Pesticides are environmental toxicants and induce the transgenerational inheritance of increased disease risk, (Table 1). Ancestral exposure of pregnant rats to the herbicide atrazine induced transgenerational increases in testis disease, prostate disease, kidney disease, a lean phenotype, and an altered age at puberty [64, 65]. DDT exposure increases obesity transgenerationally but also induces increased rates of testis, ovary, and kidney pathologies [61]. The pesticide methoxychlor, marketed as a replacement for DDT, in rats induced transgenerational increases in kidney disease and ovarian disease, which were primarily inherited through the female germ line [66]. A mixture of the insecticide permethrin and the insect repellent N, N-Diethyl-meta-toluamide (DEET) induced transgenerational increases in pubertal abnormalities, testis disease, and ovarian disease [67].

Some industrial pollutants have been investigated for their capacity to induce transgenerational increases in disease. Ancestral exposure of rats to dioxins can lead to increased kidney disease in males, pubertal abnormalities in females, and ovarian primordial follicle loss and polycystic ovary disease in F3 generation animals [68]. Exposure of zebrafish to benzo[a]pyrene, a byproduct of combustion of organic material, results in transgenerational increases in neurobehavioral abnormalities and body mass index [69].

Zebrafish ancestrally exposed to arsenic show transgenerational alterations in motor activity and increased anxiety-like behaviors [70]. Exposure of pregnant rats to arsenic resulted in transgenerational increases in testis abnormalities, reduced sperm quality, decreased adult body weight, and genotoxicity of white blood cells [71, 72], associated with DNA methylation changes and altered transcription of the IGF2 and H19 genes in testis [72]. Arsenite exposure of the nematode worm *C. elegans* resulted in alterations in sugar metabolism for six subsequent generations [73] and with decreased reproductive brood size for three generations [74].

Increased transgenerational disease has been associated with other environmental toxicants, (Table 1). Exposure of pregnant mice to ethanol vapor induces transgenerational neurological changes in the F3 generation that resemble those of Fetal Alcohol Spectrum Disorders [75]. Changes include altered ectopic intraneocortical connectivity and upregulation of Rzrβ and Id2 gene expression in the neocortex. Zebrafish exposed to methylmercury have unexposed descendants (F2 generation) that exhibit hyperactivity and a visual deficit [76]. In the crustacean *Daphnia magna*, exposure to the toxicant 5-azacytidine results in decreased body length and reduced levels of DNA methylation in non-exposed subsequent generations [77]. Endocrine disrupting chemicals can be present as natural ingredients in foods. An example is genistein, which is an estrogenic substance found in legumes and soy [78]. Treatment of fertilized quail eggs with genistein resulted in a transgenerational change in the age of sexual maturity of birds three generations later [79].

Etiology of Epigenetic Transgenerational Inheritance

In order for an environmental exposure or toxicant to induce epigenetic transgenerational inheritance, two conditions must be met. First, exposure to a toxicant must result in epigenetic changes in the germ cells (sperm or eggs) since it is the germ cells that carry molecular information to subsequent generations (Fig. 2). Second, the epigenetic changes induced in transgenerational generation animals must cause changes in gene expression in these animals or else no phenotypic changes will occur.

There are two periods during normal development when DNA methylation patterns are largely erased and reset. This epigenetic reprograming of DNA methylation occurs both immediately after fertilization in the early embryo and in developing germ cells at the time of gonadal sex determination [80]. This process allows embryonic stem cells to develop by removing epigenetic constraints to pluripotency. The well-studied exception to this is the case of imprinted genes, which retain their epigenetic DNA methylation pattern in a parent-of-origin allelic manner [81, 82]. In situations where environmentally induced epigenetic changes are inherited, some retention of these DNA

methylation patterns is thought to occur in an imprinted gene-like manner [83] (Fig. 2). Then epigenetic changes present in germ cells can transmit an altered epigenome to all cells of the subsequent developing embryo, potentially resulting in changes to gene expression that lead to an altered phenotype and disease [84] (Fig. 2).

There are many examples of exposure to toxicants leading to transgenerational epigenetic changes in germ cells, (Fig. 4 and Table 1). Altered DNA methylation of a region of DNA is termed a Differential DNA Methylated Region (DMR). If F0 generation pregnant rats were treated with vinclozolin, then sperm from the transgenerational F3 generation has been shown to have DMRs [48, 85]. Similarly, DMRs were found in transgenerational sperm after ancestral exposure of rats to a mixture of plastic-derived compounds (phthalates and BPA) [58], the dioxin TCDD [68], jet fuel hydrocarbons (JP8) [62], the herbicides atrazine [65] and glyphosate [86], the pesticides methoxychlor [66] and DDT [61, 87], a mixture of the insecticide permethrin and the insect repellent DEET [67], and the flame retardant BDE-209 [88]. In zebrafish, transgenerational sperm DMRs are found after ancestral exposure to methylmercury [76].

Other epigenetic factors, in addition to DNA methylation, can be altered in sperm transgenerationally. During spermatogenesis, the histones around which DNA is wrapped are replaced by protamines to allow DNA to be tightly compacted into the small sperm head [89]. However, there are 1–10% of histones that are retained in the sperm of most mammals [90]. These retained histones are thought to help regulate some of the early gene expression processes in the resulting embryos [91]. Studies in rats found that additional histone retention sites were present in the F3 generation sperm after pregnant F0 generation animals were treated with vinclozolin, DDT, glyphosate, or atrazine [64, 92, 93]. Therefore, histone retention in sperm is another epigenetic mechanism for transgenerational inheritance (Fig. 2). Post-translational modification of those histones retained in sperm is another epigenetic factor that can mediate transgenerational inheritance of disease. As an example, changes to methylation of histone 3 lysine 4 (H3K4me2) in mouse sperm have been associated with a transgenerational decrease in pup survival and impaired development [94]. Exposure of pregnant rats to the toxicants vinclozolin or DDT both resulted in sites of altered methylation of lysine 27 of histone 3 (H3K27me3) in transgenerational F3 generation sperm [92, 95, 96].

The expression of ncRNAs in sperm is another epigenetic factor that can be altered after exposure to endocrine disruptors [97] (Fig. 2). In studies in rats, ancestral exposure to vinclozolin induced changes in the levels of several sperm ncRNAs, including tRNA-derived small ncRNAs, namely 5′ halves of mature tRNAs, and micro-RNAs (miRNAs) [95, 98]. Similar results were found transgenerationally after ancestral exposure to DDT [96]. Transgenerational changes in ncRNA expression have been shown to occur early in germ cell development, as mice ancestrally exposed to vinclozolin have altered miRNA expression in primordial germ cells [99].

The above epigenetic factors found in sperm likely act together to pass altered phenotypes to subsequent generations [97]. Exposure to either vinclozolin or DDT induces concurrent transgenerational changes to the DNA methylation, histone retention, and ncRNA in the sperm epigenome [95, 96]. In these cases, there is evidence that RNA-directed DNA methylation and DNA methylation-directed histone retention are a part of epigenetic transgenerational inheritance [100]. The combined actions of the epigenetic factors in germ cells provide an epigenetic mechanism by which exposure to endocrine-disrupting compounds can promote the inheritance of pathologies across generations.

Epigenetic changes passed through germ cells to subsequent generations do not themselves alter phenotype. Phenotypic changes are the result of changes in gene expression. Transgenerational increases in kidney or prostate disease, or in tumor development, are the result of abnormal gene expression in the affected somatic cells. Germ cells with an altered epigenome produce embryonic stem cells that then promote epigenetic changes in all somatic cells [1, 84] (Fig. 2). These somatic cell epigenetic changes could then promote changes in gene expression that alters the phenotypes of these cells, including promoting an increased susceptibility to develop disease [101]. Therefore, in a transgenerational animal, all cell types have an altered epigenome and transcriptome. Those cell types sensitive to this alteration will have a susceptibility to develop diseases.

Several examples of transgenerational changes to gene expression following ancestral exposure to toxicants have been reported. After gestating mice were exposed to the organochlorine insecticide chlordecone, there were transgenerational changes in the transcriptome of prostates from F3 generation animals [102]. This was accompanied by an increased prostatic intraepithelial neoplasia phenotype and by histone H3K4 trimethylation (H3K4me3) and H3K27 trimethylation (H3K27me3) changes in somatic prostate cells. Similarly, ancestral exposure to vinclozolin in rats resulted in transgenerational changes to the prostate epithelial cell transcriptome and DNA methylation, associated with later-life development of prostate disease [103]. Ancestral exposure to vinclozolin also resulted in transgenerational changes to the transcriptome and epigenome of testicular Sertoli cells, associated with male infertility [84]. In female rats, both DDT and vinclozolin ancestral exposure induced transcriptome changes in the granulosa cells of the ovary, consistent with later life development of polycystic ovarian disease and reduced oocyte number [104]. This was accompanied by sites of altered DNA methylation and changes of expression of ncRNAs in the granulosa cells. In zebrafish, exposure of developing F0 generation embryos to lead resulted in F2 generation changes in brain gene expression for genes involved in physiological processes such as synaptic function and plasticity, neurogenesis, endocrine homeostasis, and epigenetic modification [105]. Ancestral exposure of zebrafish to arsenic resulted in transgenerational changes in brain-derived neurotrophic factor expression in the brain [70]. Ancestral arsenic exposure in *C. elegans* nematode worms decreased somatic cell mRNA expression of the LSD/KDM1 and spr-5 genes [74]. Therefore, the toxicant-induced epigenetic transgenerational inheritance of pathology is due to somatic cell epigenetic and transcriptome alterations that generate the phenotypes observed (Fig. 2).

A more comprehensive study of transgenerational alterations to gene expression was performed using F3 generation rats ancestrally exposed to vinclozolin [101]. The transcriptomes of 11 different organ tissues in male and female rats were evaluated and compared to those same organ tissues in F3 generation control rats ancestrally treated with vehicle. Transgenerational changes to gene expression were found in all tissues evaluated. There was minimal overlap in the genes affected between tissues, but there was considerable overlap in the physiological pathways affected by these gene expression changes. For example, both prostate and liver tissues were enriched for genes in transcription and focal adhesion processes, but the specific genes altered were not the same in each tissue [101]. Across the genome of these animals, it

was found that there existed statistically over-represented clusters of gene expression changes and that these regions, termed Epigenetic Control Regions (ECR), contained sites of altered DNA methylation (DMRs) and long ncRNA expression [95, 106]. The hypothesis is that the genes within an ECR are epigenetically regulated as a block [107]. Therefore, in one organ tissue, such as the liver, those genes that would normally be expressed from an ECR in liver cells would have altered expression, while in the prostate, a different set of genes from that same ECR (those normally expressed in the prostate) would have altered expression. These investigations all support the proposed mechanism of toxicant-induced transgenerational epimutations altering gene expression and ultimately leading to phenotypic effects, most importantly increased susceptibility for disease (Fig. 2).

Generational Toxicology

The existence of generational toxicological processes, in which the effects of toxicant exposures are seen several generations later, suggests regulatory decisions about toxicants in our society should now consider potential effects across generations. The current regulatory paradigm of evaluating experiments, where pregnant animals are treated and their direct offspring are evaluated for negative effects, may not go far enough. It is possible, with epigenetic transgenerational inheritance, that increases in disease are not seen until later generations. When pregnant F0 generation rats were treated with the herbicide glyphosate, no serious abnormalities were seen in the directly exposed F1 generation. However, dramatic increases in prostate disease, obesity, kidney disease, ovarian disease, and parturition (birth) abnormalities were seen in the F2 and F3 generations [86, 93]. Similarly, rats ancestrally exposed to the herbicide atrazine showed only a mild decrease in size in the F1 generation, but the F2 and F3 generations were found to have increased frequency of testis disease, mammary tumors, early onset puberty, motor hyperactivity, and a lean phenotype compared to controls [65]. The epigenetic transgenerational inheritance of abnormalities and increased incidence of disease after ancestral exposure to environmental toxicants should be of concern of the public and regulatory agencies for human health reasons [108].

In considering the experimental approach for regulatory agencies, animal studies should include breeding to the F3 generation to assess generational toxicity. An alternate approach would be to assess the epigenetic changes in the germ cells from the F1 generation animals. In the event germ cell epimutations exist, then the potential for generational toxicity is present. This would require additional generations to be obtained for epigenetic and pathology analysis. Although any epigenetic factor could be assessed, DNA methylation has been shown to be robust and one of the key epigenetic processes to assess. Genome-wide procedures such as bisulfite sequencing or MeDIP are optimal to assess germline epigenetic impacts. Therefore, the technology and previous literature demonstrate generational toxicity needs to be considered in the field of toxicology.

Conclusions

Research into environmentally induced epigenetic transgenerational inheritance has provided evidence for transgenerational inheritance of epimutations and phenotype changes in a wide variety of organisms [109, 110], (Fig. 4). Exposure to toxicants can induce epigenetic changes in germ cells that are passed to subsequent generations. When epimutations in the resulting embryo become imprinted-like and escape the normal processes of epigenetic reprogramming that occur during embryogenesis, then the epigenome of the embryonic stem cells is altered, which impacts all the cell types of the developing fetus and adult (Fig. 2). The altered epigenome, which can change gene expression and phenotype in all cell types in the body, increases disease susceptibility later in life. These epigenetic changes are passed to that organism's germ cells, which can be inherited by the subsequent generation. If epigenetic and phenotypic changes are passed to a generation that was never exposed to the toxicant, then epigenetic transgenerational inheritance has resulted in generational toxicology [1]. Epigenetic transgenerational inheritance of increased susceptibility to disease is an example of generational toxicity, in which toxicants affect non-exposed future generations. Governmental policies regulating toxicant exposure currently do not take generational effects into account. Future toxicity testing and regulations need to consider the effects of epigenetic transgenerational inheritance of disease and generational toxicology. A new approach that takes into consideration generational toxicology will be needed to protect our future populations.

Acknowledgements

We thank the critical insights and assistance of Dr Jennifer L.M. Thorson, as well as Dr Daniel Beck for critically reviewing the manuscript. We acknowledge Ms Amanda Quilty for editing and Ms Heather Johnson for assistance in the preparation of the manuscript. This study was supported by the John Templeton Foundation (50183 and 61174) (https://templeton.org/) grants to M.K.S.

Funding

The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest statement

None declared.

References

1. Nilsson E, Sadler-Riggleman I, Skinner MK. Environmentally induced epigenetic transgenerational inheritance of disease. *Environ Epigenet* 2018;**4**:1–13. dvy016.
2. Waddington CH. *Organisers and Genes*. Cambridge: Cambridge University Press, 1940.
3. Van Speybroeck L. From epigenesis to epigenetics: the case of C. H. Waddington. *Ann N Y Acad Sci* 2002;**981**:61–81.
4. Skinner MK. Environmental epigenetic transgenerational inheritance and somatic epigenetic mitotic stability. *Epigenet: Off J DNA Methylation Soc* 2011;**6**:838–42.
5. Holliday R, Pugh JE. DNA modification mechanisms and gene activity during development. *Science* 1975;**187**:226–32.
6. Razin A, Riggs AD. DNA methylation and gene function. *Science* 1980;**210**:604–10.
7. Turner BM. Histone acetylation as an epigenetic determinant of long-term transcriptional competence. *Cell Mol Life Sci* 1998;**54**:21–31.
8. Yaniv M. Chromatin remodeling: from transcription to cancer. *Cancer Genet* 2014;**207**:352–7.
9. Jodar M, Selvaraju S, Sendler E *et al.* The presence, role and clinical use of spermatozoal RNAs. *Hum Reprod Update* 2013;**19**:604–24.

10. Mattick JS, Lee JT. The genetic signatures of noncoding RNAs. *PLoS Genet* 2009;**5**:e1000459.
11. Schaefer M, Pollex T, Hanna K *et al.* RNA methylation by Dnmt2 protects transfer RNAs against stress-induced cleavage. *Genes Dev* 2010;**24**:1590–5.
12. Feil R. Environmental and nutritional effects on the epigenetic regulation of genes. *Mutat Res* 2006;**600**:46–57.
13. Bloomfield FH. Epigenetic modifications may play a role in the developmental consequences of early life events. *J Neurodev Disord* 2011;**3**:348–55.
14. Linner A, Almgren M. Epigenetic programming—The important first 1000 days. *Acta Paediatr* 2020;**109**:443–52.
15. Singer J, Roberts-Ems J, Riggs AD. Methylation of mouse liver DNA studied by means of the restriction enzymes msp I and hpa II. *Science* 1979;**203**:1019–21.
16. Kriaucionis S, Tahiliani M. Expanding the epigenetic landscape: novel modifications of cytosine in genomic DNA. *Cold Spring Harb Perspect Biol* 2014;**6**:a018630.
17. An J, Rao A, Ko M. TET family dioxygenases and DNA demethylation in stem cells and cancers. *Exp Mol Med* 2017;**49**:e323.
18. Mellen M, Ayata P, Heintz N. 5-hydroxymethylcytosine accumulation in postmitotic neurons results in functional demethylation of expressed genes. *Proc Natl Acad Sci U S A* 2017;**114**:E7812–E21.
19. Tang WW, Dietmann S, Irie N *et al.* A unique gene regulatory network resets the human germline epigenome for development. *Cell* 2015;**161**:1453–67.
20. Tang WW, Kobayashi T, Irie N *et al.* Specification and epigenetic programming of the human germ line. *Nat Rev Genet* 2016;**17**:585–600.
21. Reik W, Surani MA. Germline and pluripotent stem cells. *Cold Spring Harb Perspect Biol* 2015;**7**:a019422.
22. Wu TP, Wang T, Seetin MG *et al.* DNA methylation on N(6)-adenine in mammalian embryonic stem cells. *Nature* 2016;**532**:329–33.
23. Beck D, Ben Maamar M, Skinner MK. Genome-wide CpG density and DNA methylation analysis method (MeDIP, RRBS, and WGBS) comparisons. *Epigenet: Off J DNA Methylation Soc* 2021;**11**:1–13.
24. Rothbart SB, Strahl BD. Interpreting the language of histone and DNA modifications. *Biochim Biophys Acta* 2014;**1839**:627–43.
25. Taylor BC, Young NL. Combinations of histone post-translational modifications. *Biochem J* 2021;**478**:511–32.
26. Bartova E, Krejci J, Harnicarova A *et al.* Histone modifications and nuclear architecture: a review. *J Histochem Cytochem* 2008;**56**:711–21.
27. Quina AS, Buschbeck M, Di Croce L. Chromatin structure and epigenetics. *Biochem Pharmacol* 2006;**72**:1563–9.
28. Carter B, Zhao K. The epigenetic basis of cellular heterogeneity. *Nat Rev Genet* 2021;**22**:235–50.
29. Zheng ZH, Sam TW, Zeng Y *et al.* Chromatin regulation in development: current understanding and approaches. *Stem Cells Int* 2021;**2021**:8817581.
30. Wei JW, Huang K, Yang C *et al.* Non-coding RNAs as regulators in epigenetics (Review). *Oncol Rep* 2017;**37**:3–9.
31. Rong D, Sun G, Wu F *et al.* Epigenetics: roles and therapeutic implications of non-coding RNA modifications in human cancers. *Mol Ther Nucleic Acids* 2021;**25**:67–82.
32. Kornfeld JW, Bruning JC. Regulation of metabolism by long, non-coding RNAs. *Front Genet* 2014;**5**:57.
33. Pauli A, Rinn JL, Schier AF. Non-coding RNAs as regulators of embryogenesis. *Nat Rev Genet* 2011;**12**:136–49.
34. Chen Q, Yan M, Cao Z *et al.* Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. *Science* 2016;**351**:397–400.
35. Yan M, Zhai Q. Sperm tsRNAs and acquired metabolic disorders. *J Endocrinol* 2016;**230**:F13–8.
36. Chen Q, Yan W, Duan E. Epigenetic inheritance of acquired traits through sperm RNAs and sperm RNA modifications. *Nat Rev Genet* 2016;**17**:733–43.
37. Li Y. Modern epigenetics methods in biological research. *Methods* 2021;**187**:104–13.
38. Sibbritt T, Patel HR, Preiss T. Mapping and significance of the mRNA methylome. *Wiley Interdiscip Rev RNA* 2013;**4**:397–422.
39. Yue Y, Liu J, He C. RNA N6-methyladenosine methylation in post-transcriptional gene expression regulation. *Genes Dev* 2015;**29**:1343–55.
40. Fu Y, Dominissini D, Rechavi G *et al.* Gene expression regulation mediated through reversible m(6)A RNA methylation. *Nat Rev Genet* 2014;**15**:293–306.
41. Xu L, Liu X, Sheng N *et al.* Three distinct 3-methylcytidine (m(3)C) methyltransferases modify tRNA and mRNA in mice and humans. *J Biol Chem* 2017;**292**:14695–703.
42. Longenecker JZ, Gilbert CJ, Golubeva VA *et al.* Epitranscriptomics in the heart: a focus on m(6)A. *Curr Heart Fail Rep* 2020;**17**:205–12.
43. Skinner MK. What is an epigenetic transgenerational phenotype? F3 or F2. *Reprod Toxicol* 2008;**25**:2–6.
44. Blewitt ME, Vickaryous NK, Paldi A *et al.* Dynamic reprogramming of DNA methylation at an epigenetically sensitive allele in mice. *PLoS Genet* 2006;**2**:e49.
45. Waterland RA, Travisano M, Tahiliani KG. Diet-induced hypermethylation at agouti viable yellow is not inherited transgenerationally through the female. *FASEB J* 2007;**21**:3380–5.
46. Waddington CH. Canalisation of development and the inheritance of acquired characters. *Nature* 1942;**150**:563–5.
47. Anway MD, Cupp AS, Uzumcu M *et al.* Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* 2005;**308**:1466–9.
48. Nilsson E, King SE, McBirney M *et al.* Vinclozolin induced epigenetic transgenerational inheritance of pathologies and sperm epimutation biomarkers for specific diseases. *PLoS One* 2018;**13**:1–29, e0202662.
49. Nilsson EE, Anway MD, Stanfield J *et al.* Transgenerational epigenetic effects of the endocrine disruptor vinclozolin on pregnancies and female adult onset disease. *Reproduction* 2008;**135**:713–21.
50. Nilsson E, Larsen G, Manikkam M *et al.* Environmentally induced epigenetic transgenerational inheritance of ovarian disease. *PLoS One* 2012;**7**:1–18, e36129.
51. Skinner MK, Anway MD. Epigenetic transgenerational actions of vinclozolin on the development of disease and cancer. *Crit Rev Oncog* 2007;**13**:75–82.
52. Quinnies KM, Doyle TJ, Kim KH *et al.* Transgenerational effects of Di-(2-Ethylhexyl) phthalate (DEHP) on stress hormones and behavior. *Endocrinology* 2015;**156**:3077–83.
53. Rattan S, Brehm E, Gao L *et al.* Prenatal exposure to di(2-ethylhexyl) phthalate disrupts ovarian function in a transgenerational manner in female mice. *Biol Reprod* 2018;**98**:130–45.
54. Wolstenholme JT, Edwards M, Shetty SR *et al.* Gestational exposure to bisphenol a produces transgenerational changes in behaviors and gene expression. *Endocrinology* 2012;**153**:3828–38.

55. Drobna Z, Henriksen AD, Wolstenholme JT *et al.* Transgenerational effects of bisphenol A on gene expression and DNA methylation of imprinted genes in brain. *Endocrinology* 2018;**159**:132–44.
56. Lombo M, Fernandez-Diez C, Gonzalez-Rojo S *et al.* Transgenerational inheritance of heart disorders caused by paternal bisphenol A exposure. *Environ Pollut* 2015;**206**:667–78.
57. Bhandari RK, vom Saal FS, Tillitt DE. Transgenerational effects from early developmental exposures to bisphenol A or 17alpha-ethinylestradiol in medaka, Oryzias latipes. *Sci Rep* 2015;**5**:9303.
58. Manikkam M, Tracey R, Guerrero-Bosagna C *et al.* Plastics derived endocrine disruptors (BPA, DEHP and DBP) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. *PLoS One* 2013;**8**:1–18, e55387.
59. Yu CW, Luk TC, Liao VH. Long-term nanoplastics exposure results in multi and trans-generational reproduction decline associated with germline toxicity and epigenetic regulation in Caenorhabditis elegans. *J Hazard Mater* 2021;**412**:125173.
60. Chamorro-Garcia R, Sahu M, Abbey RJ *et al.* Transgenerational inheritance of increased fat depot size, stem cell reprogramming, and hepatic steatosis elicited by prenatal exposure to the obesogen tributyltin in mice. *Environ Health Perspect* 2013;**121**:359–66.
61. Skinner MK, Manikkam M, Tracey R *et al.* Ancestral dichlorodiphenyltrichloroethane (DDT) exposure promotes epigenetic transgenerational inheritance of obesity. *BMC Med* 2013;**11**:1–16.
62. Tracey R, Manikkam M, Guerrero-Bosagna C *et al.* Hydrocarbons (jet fuel JP-8) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. *Reprod Toxicol* 2013;**36**:104–16.
63. Gong Y, Xue Y, Li X *et al.* Inter- and transgenerational effects of paternal exposure to inorganic arsenic. *Adv Sci (Weinh)* 2021;**8**:2002715.
64. Thorson JLM, Beck D, Ben Maamar M *et al.* Epigenome-wide association study for atrazine induced transgenerational DNA methylation and histone retention sperm epigenetic biomarkers for disease. *PLoS One* 2020;**15**:1–29, e0239380.
65. McBirney M, King SE, Pappalardo M *et al.* Atrazine induced epigenetic transgenerational inheritance of disease, lean phenotype and sperm epimutation pathology biomarkers. *PLoS One* 2017;**12**:1–37, e0184306.
66. Manikkam M, Haque MM, Guerrero-Bosagna C *et al.* Pesticide methoxychlor promotes the epigenetic transgenerational inheritance of adult onset disease through the female germline. *PLoS One* 2014;**9**:1–19, e102091.
67. Manikkam M, Tracey R, Guerrero-Bosagna C *et al.* Pesticide and insect repellent mixture (Permethrin and DEET) induces epigenetic transgenerational inheritance of disease and sperm epimutations. *Reprod Toxicol* 2012;**34**:708–19.
68. Manikkam M, Tracey R, Guerrero-Bosagna C *et al.* Dioxin (TCDD) induces epigenetic transgenerational inheritance of adult onset disease and sperm epimutations. *PLoS One* 2012;**7**:1–15, e46249.
69. Knecht AL, Truong L, Marvel SW *et al.* Transgenerational inheritance of neurobehavioral and physiological deficits from developmental exposure to benzo[a]pyrene in zebrafish. *Toxicol Appl Pharmacol* 2017;**329**:148–57.
70. Valles S, Hernandez-Sanchez J, Dipp VR *et al.* Exposure to low doses of inorganic arsenic induces transgenerational changes on behavioral and epigenetic markers in zebrafish (Danio rerio). *Toxicol Appl Pharmacol* 2020;**396**:115002.
71. Nava-Rivera LE, Betancourt-Martinez ND, Lozoya-Martinez R *et al.* Transgenerational effects in DNA methylation, genotoxicity and reproductive phenotype by chronic arsenic exposure. *Sci Rep* 2021;**11**:8276.
72. Yin G, Xia L, Hou Y *et al.* Transgenerational male reproductive effect of prenatal arsenic exposure: abnormal spermatogenesis with Igf2/H19 epigenetic alteration in CD1 mouse. *Int J Environ Health Res* 2021;**7**:1–13.
73. Gu C, Wen Y, Wu L *et al.* Arsenite-induced transgenerational glycometabolism is associated with up-regulation of H3K4me2 via inhibiting spr-5 in caenorhabditis elegans. *Toxicol Lett* 2020;**326**:11–7.
74. Yu CW, Liao VH. Transgenerational reproductive effects of arsenite are associated with H3K4 dimethylation and SPR-5 downregulation in caenorhabditis elegans. *Environ Sci Technol* 2016;**50**:10673–81.
75. Abbott CW, Rohac DJ, Bottom RT *et al.* Prenatal ethanol exposure and neocortical development: a transgenerational model of FASD. *Cereb Cortex* 2018;**28**:2908–21.
76. Carvan M Jr, Kalluvila TA, Klingler RH *et al.* Mercury-induced epigenetic transgenerational inheritance of abnormal neurobehavior is correlated with sperm epimutations in zebrafish. *PLoS One* 2017;**12**:1–26, e0176155.
77. Vandegehuchte MB, Lemiere F, Vanhaecke L *et al.* Direct and transgenerational impact on Daphnia magna of chemicals with a known effect on DNA methylation. *Comp Biochem Physiol C Toxicol Pharmacol* 2010;**151**:278–85.
78. Patisaul HB, Melby M, Whitten PL *et al.* Genistein affects ER beta- but not ER alpha-dependent gene expression in the hypothalamus. *Endocrinology* 2002;**143**:2189–97.
79. Leroux S, Gourichon D, Leterrier C *et al.* Embryonic environment and transgenerational effects in quail. *Genet Sel Evol* 2017;**49**:14.
80. Smith ZD, Chan MM, Mikkelsen TS *et al.* A unique regulatory phase of DNA methylation in the early mammalian embryo. *Nature* 2012;**484**:339–44.
81. Constancia M, Pickard B, Kelsey G *et al.* Imprinting mechanisms. *Genome Res* 1998;**8**:881–900.
82. John RM, Lefebvre L. Developmental regulation of somatic imprints. *Differentiation* 2011;**81**:270–80.
83. Skinner MK, Manikkam M, Guerrero-Bosagna C. Epigenetic transgenerational actions of environmental factors in disease etiology. *Trends Endocrinol Metab* 2010;**21**:214–22.
84. Guerrero-Bosagna C, Savenkova M, Haque MM *et al.* Environmentally induced epigenetic transgenerational inheritance of altered sertoli cell transcriptome and epigenome: molecular etiology of male infertility. *PLoS One* 2013;**8**:1–12, e59922.
85. Guerrero-Bosagna C, Settles M, Lucker B *et al.* Epigenetic transgenerational actions of vinclozolin on promoter regions of the sperm epigenome. *PLoS One* 2010;**5**:1–17, e13100.
86. Kubsad D, Nilsson EE, King SE *et al.* Assessment of glyphosate induced epigenetic transgenerational inheritance of pathologies and sperm epimutations: generational toxicology. *Sci Rep* 2019;**9**:6372.
87. King SE, McBirney M, Beck D *et al.* Sperm epimutation biomarkers of obesity and pathologies following DDT induced epigenetic transgenerational inheritance of disease. *Environ Epigenet* 2019;**5**:1–15. dvz008.
88. Hsu P-C, Li Z-K, Lai C-S *et al.* Transgenerational effects of BDE-209 on male reproduction in F3 offspring rats. *Chemosphere* 2021;**272**:129829.

89. Bao J, Bedford MT. Epigenetic regulation of the histone-to-protamine transition during spermiogenesis. *Reproduction* 2016;**151**:R55–70.
90. Rathke C, Baarends WM, Awe S *et al.* Chromatin dynamics during spermiogenesis. *Biochim Biophys Acta* 2014;**1839**: 155–68.
91. Ihara M, Meyer-Ficca ML, Leu NA *et al.* Paternal poly (ADP-ribose) metabolism modulates retention of inheritable sperm histones and early embryonic gene expression. *PLoS Genet* 2014;**10**:e1004317.
92. Ben Maamar M, Sadler-Riggleman I, Beck D *et al.* Epigenetic transgenerational inheritance of altered sperm histone retention sites. *Sci Rep* 2018;**8**:5308, 1–10.
93. Ben Maamar M, Beck D, Nilsson EE *et al.* Epigenome-wide association study for glyphosate induced transgenerational sperm DNA methylation and histone retention epigenetic biomarkers for disease. *Epigenet: Off J DNA Methylation Soc* 2020;**9**: 1–18.
94. Siklenka K, Erkek S, Godmann M *et al.* Disruption of histone methylation in developing sperm impairs offspring health transgenerationally. *Science* 2015;**350**:1–14. aab2006.
95. Ben Maamar M, Sadler-Riggleman I, Beck D *et al.* Alterations in sperm DNA methylation, non-coding RNA expression, and histone retention mediate vinclozolin-induced epigenetic transgenerational inheritance of disease. *Environ Epigenet* 2018;**4**: 1–19. dvy010.
96. Skinner MK, Ben Maamar M, Sadler-Riggleman I *et al.* Alterations in sperm DNA methylation, non-coding RNA and histone retention associate with DDT-induced epigenetic transgenerational inheritance of disease. *Epigenet Chromatin* 2018;**11**: 8, 1–24.
97. Larriba E, Del Mazo J. Role of non-coding RNAs in the transgenerational epigenetic transmission of the effects of reprotoxicants. *Int J Mol Sci* 2016;**17**:452.
98. Schuster A, Skinner MK, Yan W. Ancestral vinclozolin exposure alters the epigenetic transgenerational inheritance of sperm small noncoding RNAs. *Environ Epigenet* 2016;**2**:dvw001.
99. Brieno-Enriquez MA, Garcia-Lopez J, Cardenas DB *et al.* Exposure to endocrine disruptor induces transgenerational epigenetic deregulation of microRNAs in primordial germ cells. *PLoS One* 2015;**10**:e0124296.
100. Beck D, Ben Maamar M, Skinner MK. Integration of sperm ncRNA-directed DNA methylation and DNA methylation-directed histone retention in epigenetic transgenerational inheritance. *Epigenet Chromatin* 2021;**14**:6.
101. Skinner MK, Manikkam M, Haque MM *et al.* Epigenetic transgenerational inheritance of somatic transcriptomes and epigenetic control regions. *Genome Biol* 2012;**13**:R91.
102. Legoff L, D'Cruz SC, Lebosq M *et al.* Developmental exposure to chlordecone induces transgenerational effects in somatic prostate tissue which are associated with epigenetic histone trimethylation changes. *Environ Int* 2021;**152**:106472.
103. Klukovich R, Nilsson E, Sadler-Riggleman I *et al.* Environmental toxicant induced epigenetic transgenerational inheritance of prostate pathology and stromal-epithelial cell epigenome and transcriptome alterations: ancestral origins of prostate disease. *Sci Rep* 2019;**9**:1–17.
104. Nilsson E, Klukovich R, Sadler-Riggleman I *et al.* Environmental toxicant induced epigenetic transgenerational inheritance of ovarian pathology and granulosa cell epigenome and transcriptome alterations: ancestral origins of polycystic ovarian syndrome and primary ovarian insufficiency. *Epigenet: Off J DNA Methylation Soc* 2018;**13**:875–95.
105. Meyer DN, Crofts EJ, Akemann C *et al.* Developmental exposure to Pb2+ induces transgenerational changes to zebrafish brain transcriptome. *Chemosphere* 2020;**244**:125527.
106. Haque MM, Nilsson EE, Holder LB *et al.* Genomic Clustering of differential DNA methylated regions (epimutations) associated with the epigenetic transgenerational inheritance of disease and phenotypic variation. *BMC Genomics* 2016;**17**:1–13.
107. Senmatsu S, Hirota K. Roles of lncRNA transcription as a novel regulator of chromosomal function. *Genes Genet Syst* 2020;**95**:213–23.
108. Xavier MJ, Roman SD, Aitken RJ *et al.* Transgenerational inheritance: how impacts to the epigenetic and genetic information of parents affect offspring health. *Hum Reprod Update* 2019;**25**:518–40.
109. Hanson MA, Skinner MK. Developmental origins of epigenetic transgenerational inheritance. *Environ Epigenet* 2016;**2**:1–9. dvw002.
110. Skinner MK, Nilsson E. Epigenetic transgenerational inheritance across species. *Encycl Reprod 2nd Ed* 2018;**3**:442–5.

"Epigenetics and Systems Biology"

Spring 2023 (Odd Years) – Course Syllabus
Biol 476/576 Undergraduate/Graduate Course (3 Credit)
SLN: (476) – 09358, (576) – 09359
Time - Tuesday and Thursday 10:35 am-11:50 am
Course Lectures in person and on Canvas/Panopto and Discussion Sessions in person and on WSU Zoom for all campuses (Hybrid Course)
Room – CUE 418
Course Director - Michael Skinner, Abelson Hall 507, 335-1524, skinner@wsu.edu
Co-Instructor – Eric Nilsson, Abelson Hall 507, 225-1835, nilsson@wsu.edu
Learning Objective - The objective of the course is to learn the concept and critical role of systems to understand molecular, cell, development, physiology and evolutionary aspects of biology with a focus on the role of epigenetics in systems biology.
Schedule/Lecture Outline –

Week 1	January 10 & 12	Systems Biology (History/ Definitions/ Theory)
Week 2	January 17 & 19	Systems Biology (Networks & Emergence)
Week 3	January 24 & 26	Systems Biology (Components: DNA to Phenotype)
Week 4	Jan 31 & Feb 2	Systems Biology (Genomics / Technology)
Week 5	February 7 & 9	Epigenetics (History / Molecular Processes)
Week 6	February 14 & 16	Epigenetics (Molecular Processes & Integration)
Week 7	February 21 & 23	Epigenetics (Genomics and Technology)
Week 8	Feb 28 & March 2	Cell & Developmental Biology
Week 9	March 7 & 9	Epigenetics of Cell & Developmental Biology (& Midterm Exam)
Week 10	**March 13 – 17**	**Spring Break**
Week 11	March 21 & 23	Environmental Impact on Biology
Week 12	March 28 & 30	Environmental Epigenetics
Week 13	April 4 & 6	Disease Etiology
Week 14	April 11 & 13	Epigenetics & Disease Etiology
Week 15	April 18 & 20	Evolutionary Biology & Genetics
Week 16	April 25 & 27	Epigenetics & Evolutionary Biology
Week 17	May 2 & 4	Grant Review/ Study Section Meeting (& Final Exam)

Spring 2023 – Epigenetics and Systems Biology
Lecture Outline (Systems Biology)
Michael K. Skinner – Biol 476/576
Weeks 11 and 12 (March 2023)

Environmental Epigenetics

- Environmental Impacts on Biology
- Environment and Phenotype Variation
- Environmental Factors
- Environmental Epigenetics and Twin Studies
- Early life Exposures and Developmental Effects
- Nutrition and Epigenetics
- Environmental Toxicants and Epigenetics
- Environmental Induced Epigenetic Transgenerational Inheritance

Required Reading

Nilsson EE, Ben Maamar M, Skinner MK. Role of epigenetic transgenerational inheritance in generational toxicology. Environ Epigenet. 2022 Feb 16;8(1):dvac001. (PMID: 35186326)

Books (Reserve in Library)

Scott F. Gilbert and David Epel (2009) Ecological Developmental Biology. Sinauer Associates Inc. Sunderland, Massachusetts.

E-Book: Craig and Wong (2011) Epigenetics: A Reference Manual. Caister Academic Press. ISBN-13: 978-1904455882.

Spring 2023 – Epigenetics and Systems Biology
Discussion Session (Environmental Epigenetics)
Michael K. Skinner – Biol 476/576
Week 11 (March 23)

Environmental Epigenetics

Primary Papers

1. Duncan GE, et al. (2022) Sci Rep. 12(1):20166. (PMID: 36424439)
2. McGowan et al., (2009) Nat Neurosci. 12(3):342-8. (PMID: 19234457)
3. Burdge et al., (2009) J Nutr. 139(6):1054-60. (PMID: 19339705)

Discussion

Student 25 – Ref #1 above

- Why are twin studies useful for epigenetic studies?
- Does the data support an environmental impact on the human epigenome and disease?
- What is the application of these epigenetic changes?

Student 26 – Ref #2 above

- What mechanism is proposed for early life effects on brain function?
- Is NGF1 the only gene effected?
- What is the impact of these epigenetic changes?

Student 27 – Ref #3 above

- How does folic acid effect epigenetics?
- Does diet effect epigenetic programming?
- What happens if you have too much folate?

Spring 2023 – Epigenetics and Systems Biology
Discussion Session (Environmental Epigenetics)
Michael K. Skinner – Biol 476/576
Week 12 (March 30)

Environmental Epigenetics

Primary Papers

1. Ben Maamar, et al. (2018) Environmental Epigenetics 26;4(2):dvy010, 1-19. (PMID: 29732173)
2. Ben Maamar, et al. (2019) Developmental Biology 445: 280-293. (PMID: 30500333)
3. Cao N, et al. (2022) Circulation. 2022 Oct 4;146(14):1082-1095. (PMID: 36004643)

Discussion

Student 28 – Ref #1 above

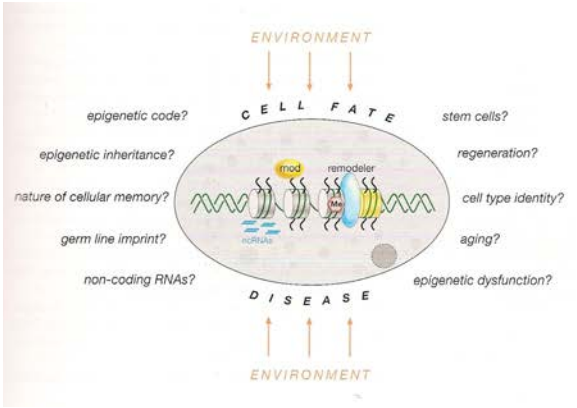
- What is the experimental design?
- What epigenetic technologies and alterations were investigated?
- What is the primary conclusion of the study?

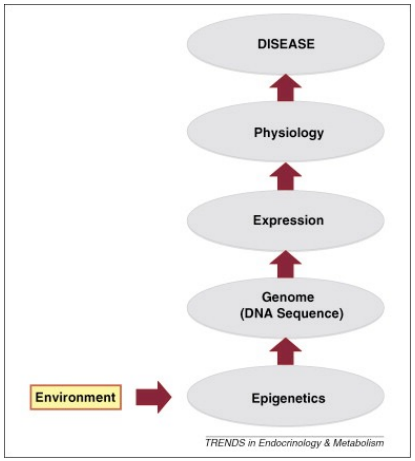
Student 29 – Ref #2 above

- What is the experimental design?
- What were the developmental origins of the sperm epimutations?
- What conclusions on the development of the sperm epimutations are made?

Student 30 – Ref #3 above

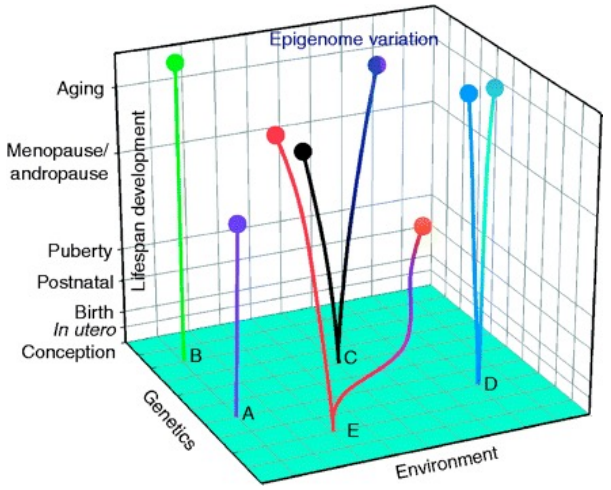
- What exposure was used?
- What transgenerational disease was observed?
- What treatment inhibited the transgenerational effect?





Agents of developmental plasticity

- Temperature
- Nutrition
- Pressure and gravity
- Light
- The presence of dangerous conditions (predators or stress)
- The presence or absence of conspecifics (other members of the same species)



Environmental Epigenetics
(Phenotypic Variation)

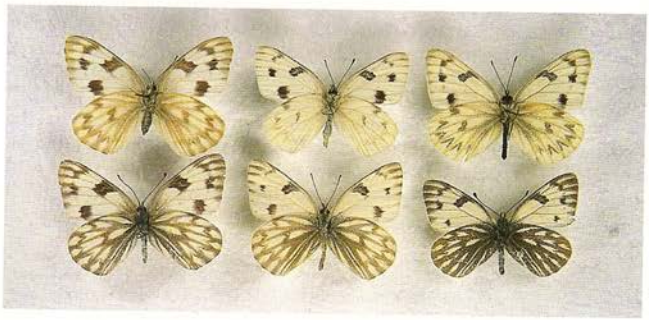


FIGURE 1.5 Polyphenic variation in *Pontia* (Pieridae) butterflies. The top row shows summer morphs: *P. protodice* female (left) and male (center), *P. occidentalis* male (right). The bottom row shows spring morphs, which have a more highly pigmented ventral hindwing: *P. protodice* female (left) and male (center), *P. occidentalis* male (right). (Photograph courtesy of T. Valente.)

FIGURE 2.8 Hormonal regulation mediates the environmentally controlled pigmentation of *Araschnia*. In the wild, different generations experience significantly different photoperiods. In the short photoperiod (below the critical day length), there is no pulse of 20-hydroxyecdysone (20E) during early pupation, and the spring form of the butterfly is generated. When these spring butterflies mate, the larvae experience a long photoperiod and generate the summer pigmentation. In the laboratory, injections of 20E at different times during pupation can induce both phenotypes, as well as intermediate phenotypes not seen in the wild. (From Nijhout 2003; photographs courtesy of H. F. Nijhout.)

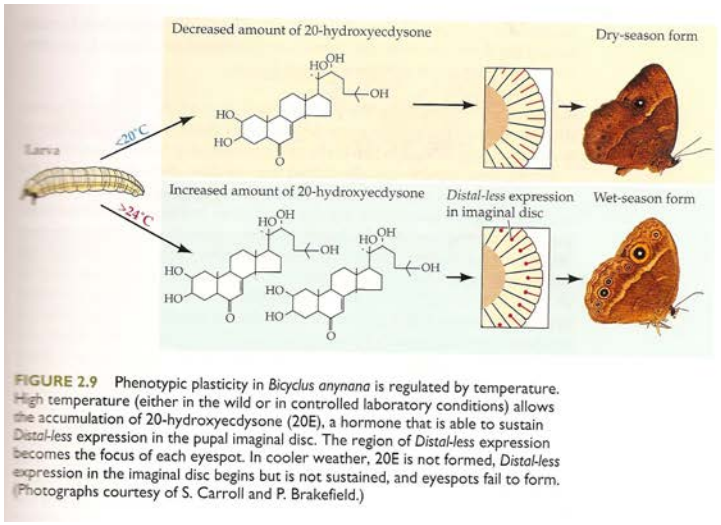


FIGURE 2.9 Phenotypic plasticity in *Bicyclus anynana* is regulated by temperature. High temperature (either in the wild or in controlled laboratory conditions) allows the accumulation of 20-hydroxyecdysone (20E), a hormone that is able to sustain *Distal-less* expression in the pupal imaginal disc. The region of *Distal-less* expression becomes the focus of each eyespot. In cooler weather, 20E is not formed, *Distal-less* expression in the imaginal disc begins but is not sustained, and eyespots fail to form. (Photographs courtesy of S. Carroll and P. Brakefield.)

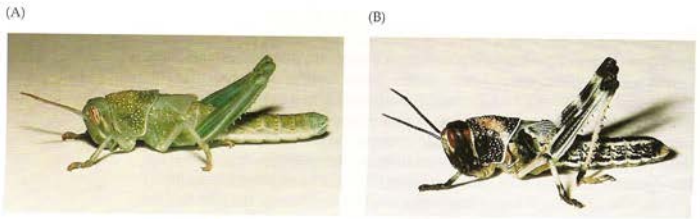


FIGURE 1.3 Density-induced polyphenism in the desert (or "plague") locust *Schistocerca gregaria*. (A) The low-density morph has green pigmentation and miniature wings. (B) Triggered by crowding, the high-density morph develops with deep pigmentation and wing and leg development suitable for migration. (From Tawfik et al. 1999, courtesy of S. Tanaka.)

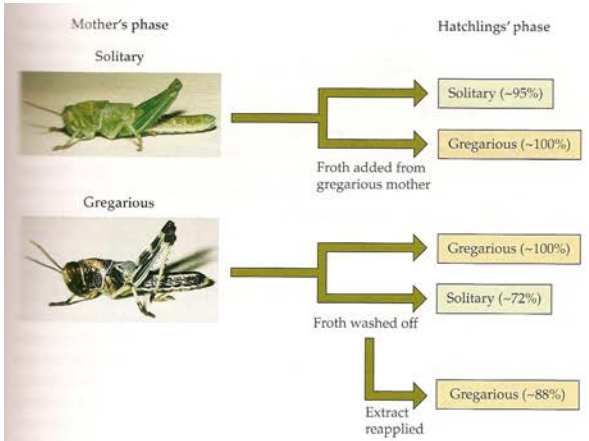


FIGURE 2.18 Summary of experiments showing that the source of the gregarizing agent in *Schistocerca* is an aqueous substance found in the froth surrounding the egg masses of gregarious females. (After McCaffery and Simpson 1998 and Simpson and Sword, in press.)

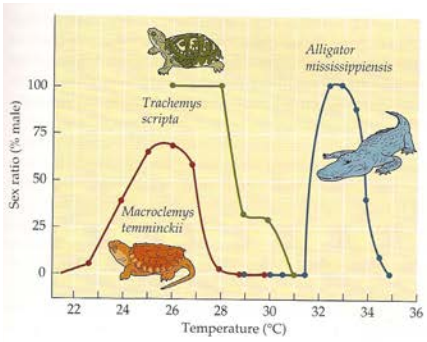


FIGURE 1.6 Temperature-dependent sex determination in three different reptilian species: the American alligator (*Alligator mississippiensis*), the red-eared slider turtle (*Trachemys scripta*), and the alligator snapping turtle (*Macroclemys temminckii*). (After Crain and Guillette 1998.)

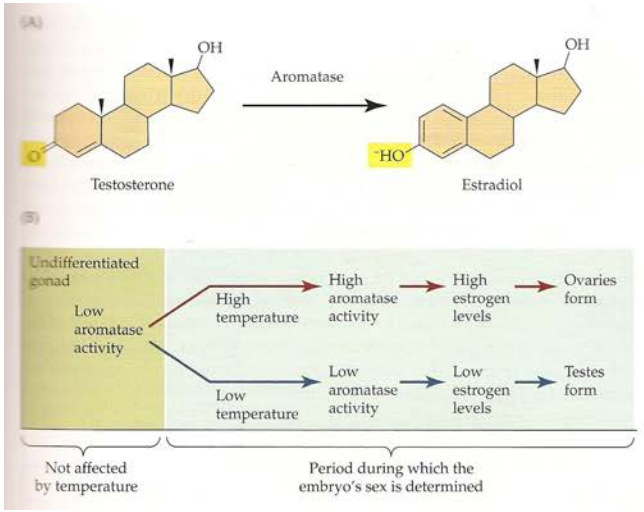
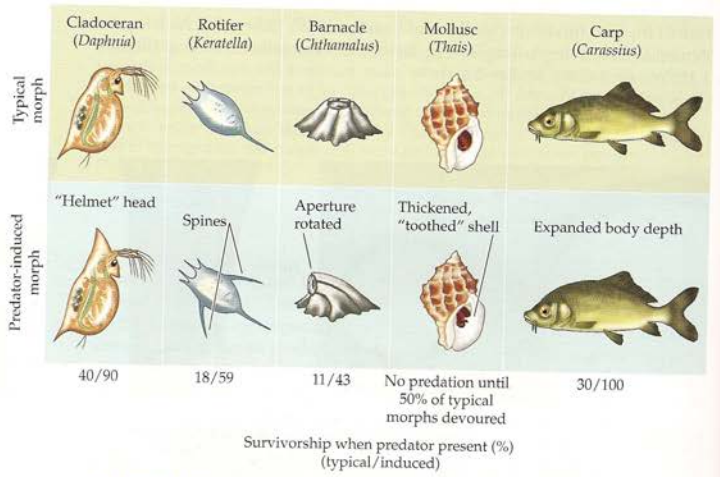
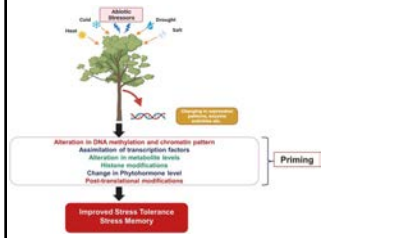


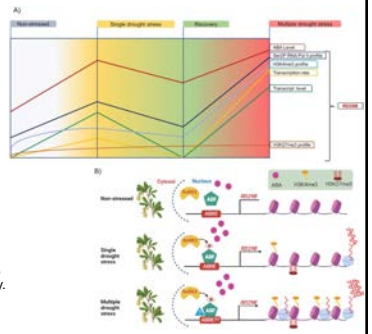
FIGURE 1.8 Gyne (reproductive queen) and worker of the ant *Pheidologeton*. This picture shows the remarkable dimorphism between the large queen and the small worker (seen near the queen's antennae). The difference between these two sisters involves larval feeding and juvenile hormone synthesis. (Photograph © Mark W. Moffett/Minden Pictures.)



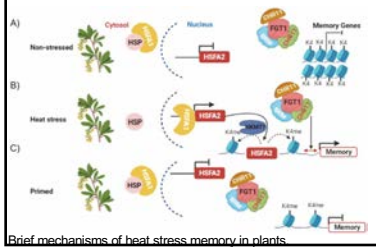
Understanding plant stress memory response for abiotic stress resilience: Molecular insights and prospects.
Sharma M, Kumar P, Verma V, Sharma R, Bhargava B, Irfan M. Plant Physiol Biochem. 2022 May 15;179:10-24.



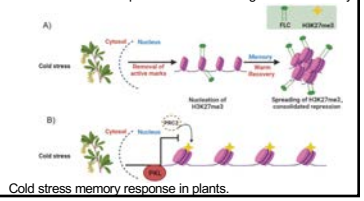
Outline of various plant responses to the different abiotic stressors to improve stress management that indicate priming and stress memory.



Role of RD29Btranscript mechanism in drought stress memory.



Brief mechanisms of heat stress memory in plants.



Cold stress memory response in plants.

Adapting to climate with limited genetic diversity: Nucleotide, DNA methylation and microbiome variation among populations of the social spider Stegodyphus dumicola.
Aagaard A, Liu S, Tregenza T, Braad Lund M, Schramm A, Verhoeven KJF, Bechsgaard J, Bilde T. Mol Ecol. 2022 Nov;31(22):5765-5783.

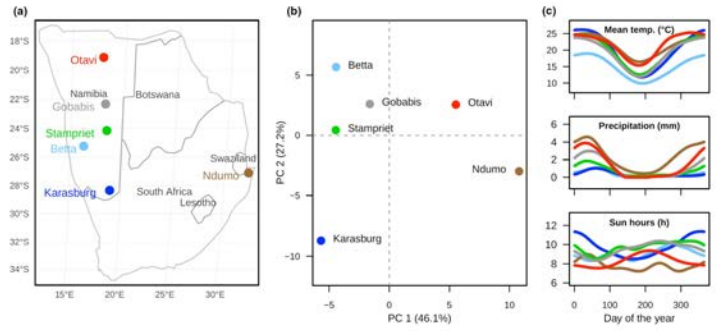


FIGURE 1 (a) Map of southern Africa showing the locations of social spider populations. (b) Climatic separation of the geographical locations on the two main environmental axes from the PCA (see details in Figures S1–S3 and Table S2). (c) Yearly variation in three climatic variables; top: Mean temperature, Centre: Precipitation, bottom: Daily hours of direct sun.

Environmental Epigenetics
(Historic Observation)

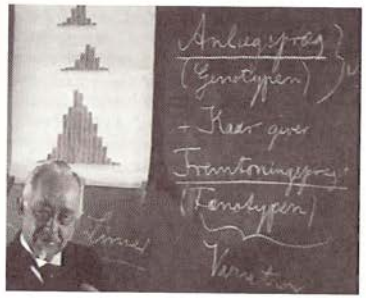
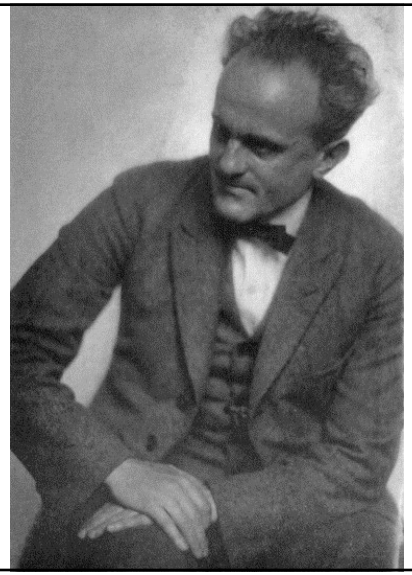
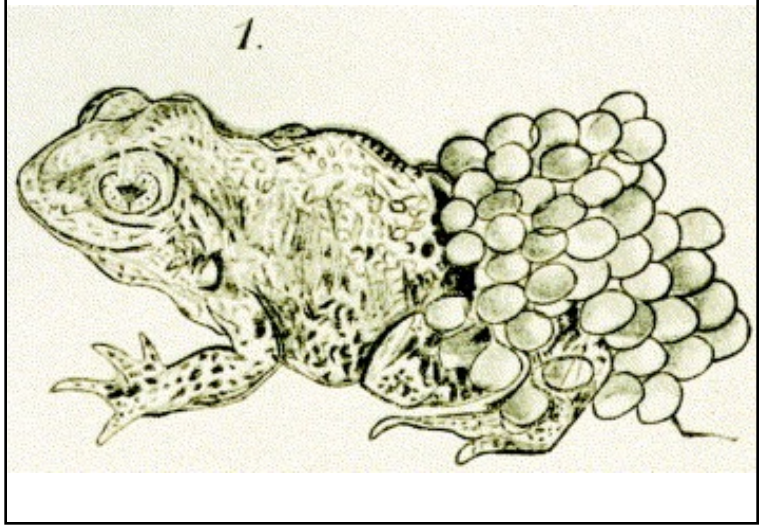


FIGURE 1.2 One hundred years ago, Wilhelm Johannsen noted that the phenotype is the product of both the genome and environmental circumstances. Here he writes on the board that *Anlaegspraeg* (genotype) + *Kaar* (Danish for "conditions" or "circumstances") gives *Fremtonigspraeg* (phenotype). (Photograph from a movie of Professor Johannsen at http://www.wjc.ku.dk/library/video/original.avi.)

Did Paul Kammerer Discover Epigenetic Inheritance? A Modern Look at the Controversial Midwife Toad Experiments





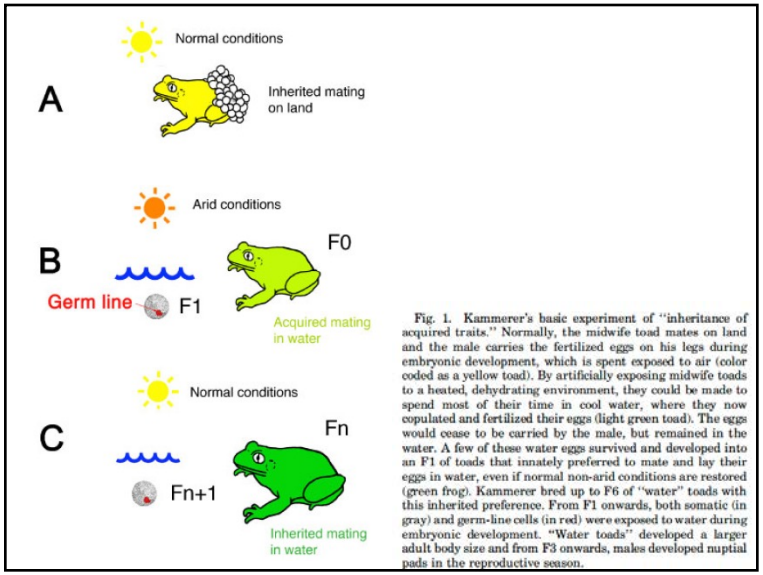
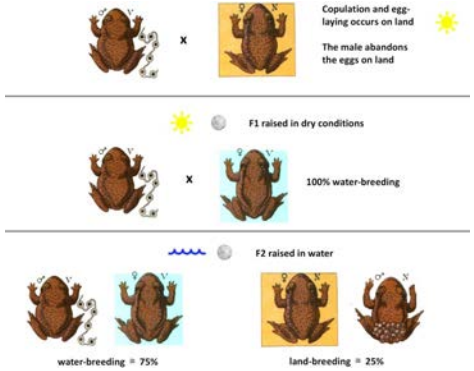


Fig. 1. Kammerer's basic experiment of "inheritance of acquired traits." Normally, the midwife toad mates on land and the male carries the fertilized eggs on his legs during embryonic development, which is spent exposed to air (color coded as a yellow toad). By artificially exposing midwife toads to a heated, dehydrating environment, they could be made to spend most of their time in cool water, where they now copulated and fertilized their eggs (light green toad). The eggs would cease to be carried by the male, but remained in the water. A few of these water eggs survived and developed into an F1 of toads that innately preferred to mate and lay their eggs in water, even if normal non-arid conditions are restored (green frog). Kammerer bred up to F6 of "water" toads with this inherited preference. From F1 onwards, both somatic (in gray) and germ-line cells (in red) were exposed to water during embryonic development. "Water toads" developed a larger adult body size and from F3 onwards, males developed nuptial pads in the reproductive season.

An Epigenetic Perspective on the Midwife Toad Experiments of Paul Kammerer (1880-1926).
J Exp Zool B Mol Dev Evol. 2017 Jan;328(1-2):179-192.
Vargas AO, Krabichler Q, Guerrero-Bosagna C.

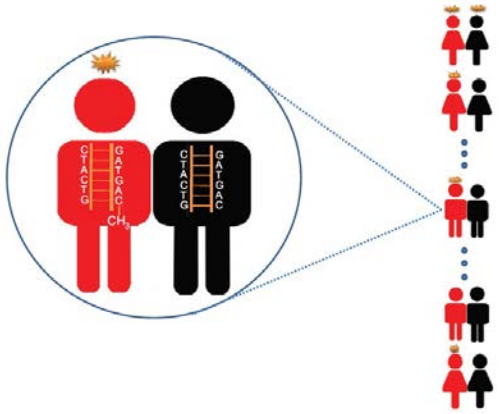


Environmental Impact on Biology

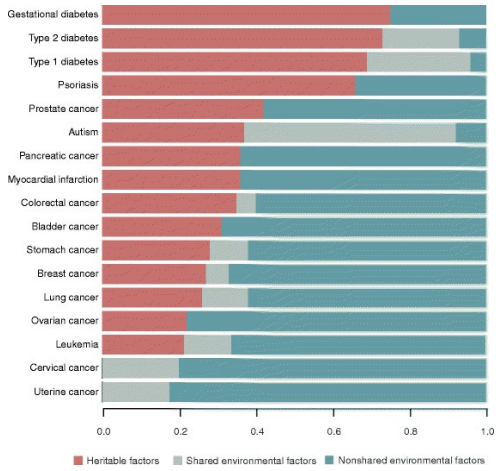
- Regional Disease Frequencies
- Low Frequency of Genetic Component of Disease (GWAS)
- Increases In Disease Frequencies
- Identical Twins and Variable Disease Frequency
- Environmental Exposures and Disease
- Evolutionary Regional Differences and Rapid Induction

Environmental Epigenetics (Twin Studies)

Twin methodology in epigenetic studies.
J Exp Biol. 2015 Jan 1;218(Pt 1):134-9.
Tan Q, Christiansen L, von Bornemann Hjelmborg J, Christensen K.



Epigenetics of discordant monozygotic twins: implications for disease.
Castillo-Fernandez JE, Spector TD, Bell JT.
Genome Med. 2014 Jul 31;6(7):60.



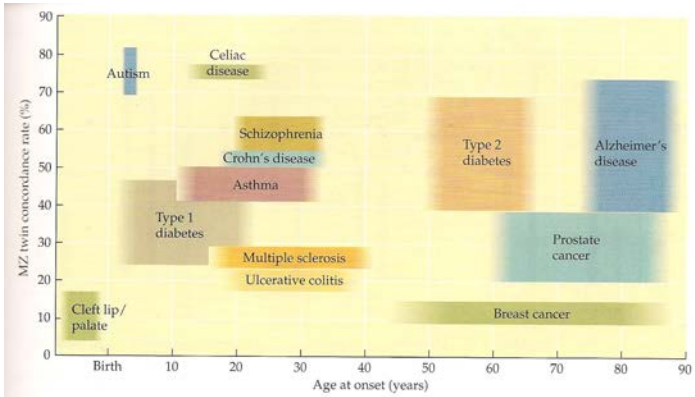


FIGURE 7.14 Age of disease onset and concordance of occurrence among monozygotic (MZ) twins for a number of pathologies. The concordance rate reflects the probability that both twins will be affected; a low rate indicates that often one twin has the disease and the other does not. Note that there is no correlation between age of onset and the degree of concordance. (After Petronis 2006.)

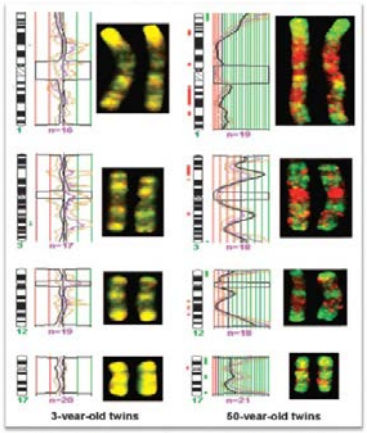


Fig. 5. Mapping chromosomal regions with differential DNA methylation in MZ twins by using comparative genomic hybridization for methylated DNA. Competitive hybridization onto normal metaphase chromosomes of the AIMS products generated from 3- and 50-year-old twin pairs. Examples of the hybridization of chromosomes 1, 3, 12, and 17 are displayed. The 50-year-old twin pair shows abundant changes in the pattern of DNA methylation observed by the presence of green and red signals that indicate hypermethylation and hypomethylation events, whereas the 3-year-old twins have a very similar distribution of DNA methylation indicated by the presence of the yellow color obtained by equal amounts of the green and red dyes. Significant DNA methylation changes are indicated as thick red and green blocks in the ideograms (Reproduced from Fraga et al. 2005)

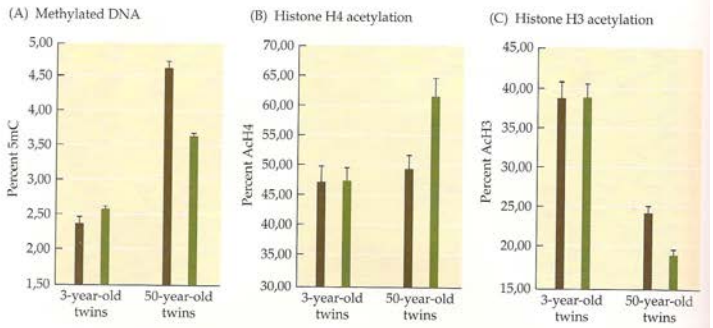
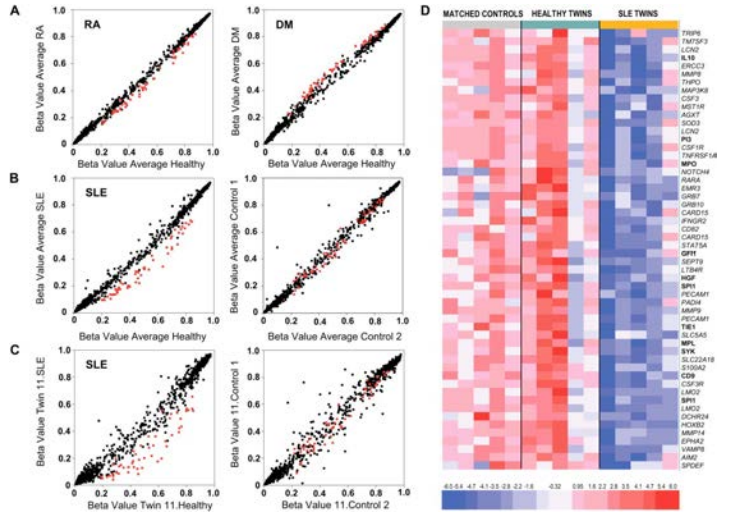


FIGURE 7.15 Monozygotic twins start off with identical amounts of (A) methylated DNA, (B) histone H4 acetylation, and (C) histone H3 acetylation. However, in older pairs, the patterns of methylation and acetylation have become discordant. (After Fraga et al. 2005.)

Changes in the pattern of DNA methylation associate with twin discordance in systemic lupus erythematosus.

Javierre BM, Fernandez AF, Richter J, Al-Shahrour F, Martin-Subero JI, Rodriguez-Ubreva J, Berdasco M, Fraga MF, O'Hanlon TP, Rider LG, Jacinto FV, Lopez-Longo FJ, Dopazo J, Forn M, Peinado MA, Carreño L, Sawalha AH, Harley JB, Siebert R, Esteller M, Miller FW, Ballestar E.

Genome Res. 2010 Feb;20(2):170-9.



Identical twins doubly exchanged at birth: a case report of genetic and environmental influences on the adult epigenome.

Segal NL, Montoya YS, Loke YJ, Craig JM.
Epigenomics. 2017 Jan;9(1):5-12.

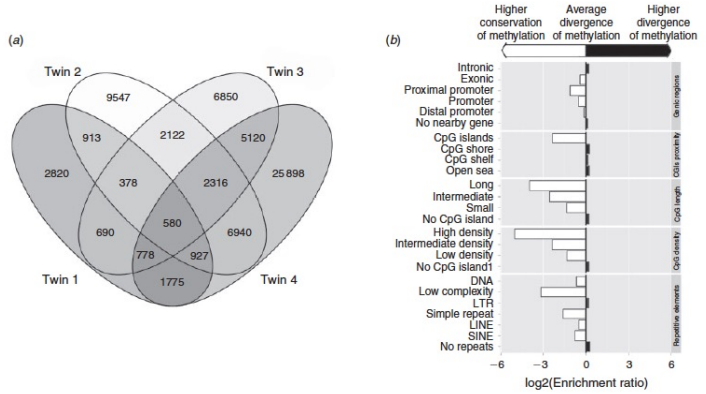
Executive summary

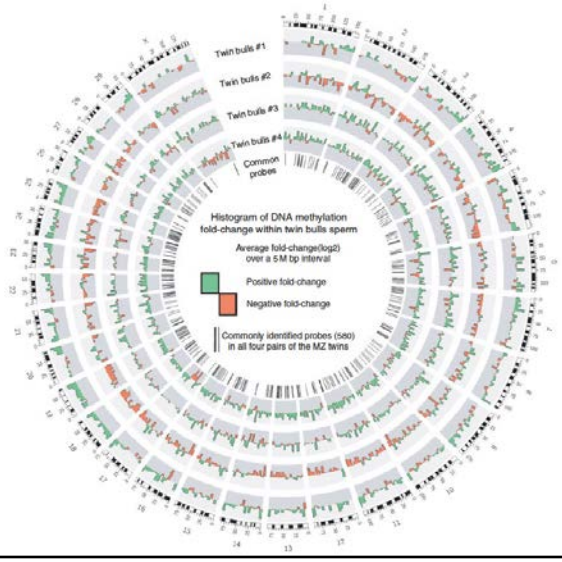
- Monozygotic twins reared apart (MZA) are good models for studying the influence of pre- and postnatal environments on epigenetics, while controlling for shared genetics.
- Genetic and intrauterine environmental factors appeared to have a stronger influence on DNA methylation than rearing environment, with the latter being very different for the two twin pairs.
- The largest effects of the rearing environment on DNA methylation within our two MZA pairs involve genes relevant to immune response and cell death.

Genome-wide analysis of sperm DNA methylation from monozygotic twin bulls.

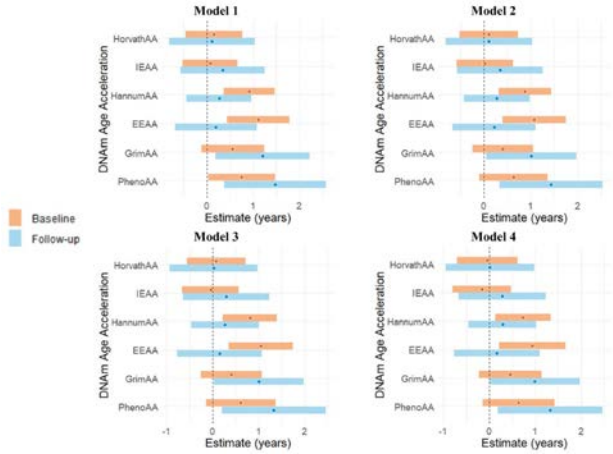
Reprod Fertil Dev. 2016 Jan 12 [Epub ahead of print]

Shojaei Saadi HA, Fournier É, Vigneault C, Blondin P, Bailey J, Robert C.



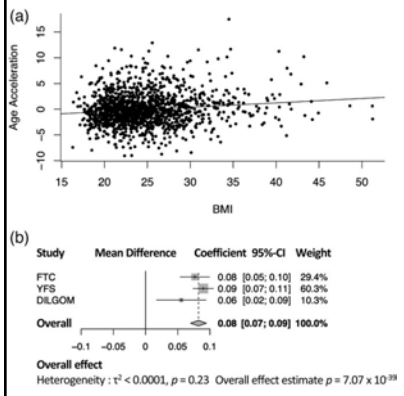


Association between depression and epigenetic age acceleration: A co-twin control study.
Liu C, Wang Z, Hui Q, Goldberg J, Smith NL, Shah AJ, Murrah N, et al.
Depress Anxiety. 2022 Jun 27. doi: 10.1002/da.23279. Online ahead of print.

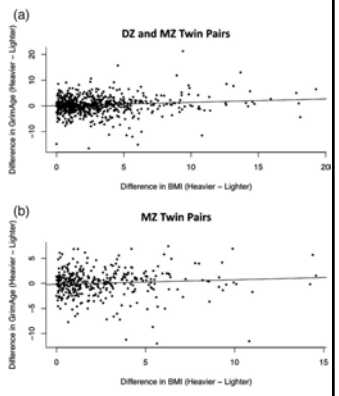


Within-pair association of BDI-II total score (per 10 units higher score) with DNAm age acceleration (in years) at the baseline and at the follow-up visit, using within-twin effect models.

BMI is positively associated with accelerated epigenetic aging in twin pairs discordant for body mass index
Lundgren S, Kuitunen S, Pietiläinen KH, Hurme M, et al.
J Intern Med. 2022 Oct;292(4):627-640.

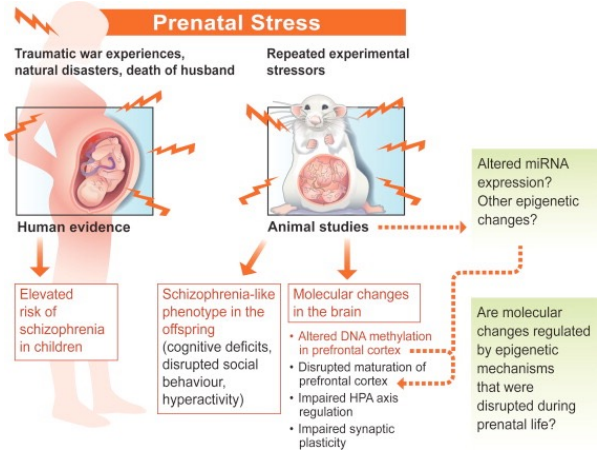


Body mass index (BMI) associates with age acceleration



Difference in body mass index (BMI) is related with the difference in GrimAge within twin pairs.

Environmental Epigenetics
(Early Life History Exposures)



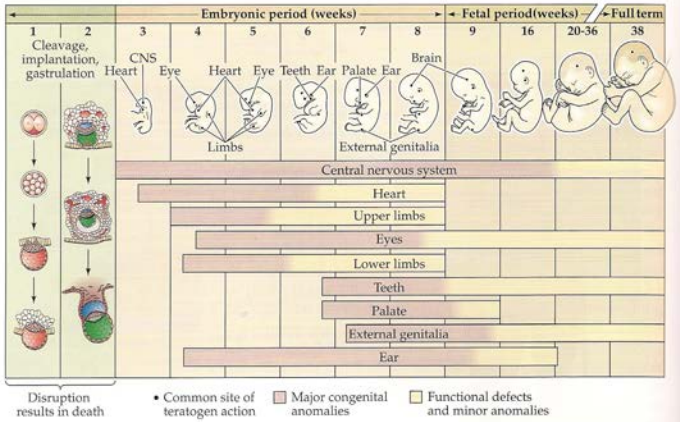


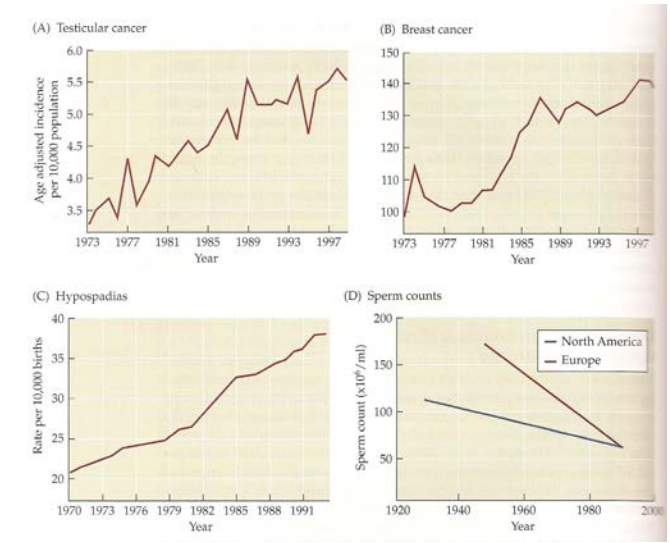
FIGURE 5.2 Periods (weeks of gestation) and degrees of sensitivity of embryonic organs to teratogens. (After Moore and Persaud 1993.)

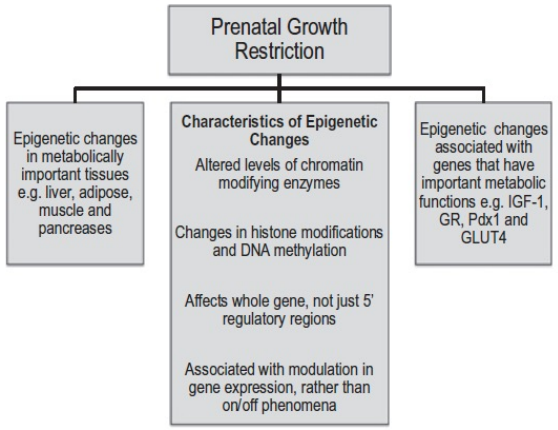
TABLE 5.1 Some agents thought to disrupt human fetal development[a]

DRUGS AND CHEMICALS	IONIZING RADIATION (X-RAYS)
Alcohol	HYPERTHERMIA (FEVER)
Aminoglycosides (Gentamycin)	INFECTIOUS MICROORGANISMS
Aminopterin	Coxsackie virus
Antithyroid agents (PTU)	Cytomegalovirus
Bromine	Herpes simplex
Cortisone	Parvovirus
Diethylstilbesterol (DES)	Rubella (German measles)
Lead	*Toxoplasma gondii* (toxoplasmosis)
Methylmercury	*Treponema pallidum* (syphilis)
Penicillamine	METABOLIC CONDITIONS IN THE MOTHER
Retinoic acid (Isotretinoin, Accutane)	Autoimmune disease (including Rh incompatibility)
Streptomycin	Diabetes
Tetracycline	Dietary deficiencies, malnutrition
Thalidomide	Phenylketonuria
Trimethadione	
Valproic acid	
Warfarin	

Source: Adapted from Opitz 1991.

[a]This list includes known and possible teratogenic agents and is not exhaustive.





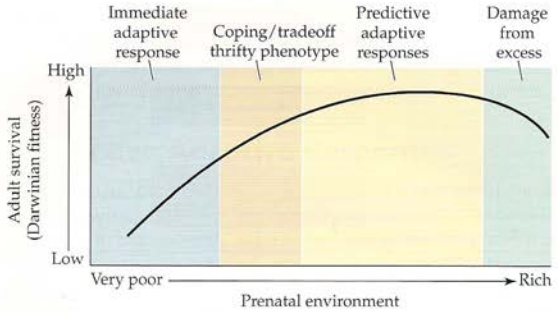


FIGURE 7.7 Developmental responses and fitness. Mutations or severe environmental disturbances can disrupt development and cause *immediate responses* to the injury or defect. Less extreme disturbances can lead to *predictive adaptive responses* (PARs), including the induction of thrifty phenotypes. Within the normal range of variation, an individual's developmental trajectory will be conferred by the actions of PARs. (After Gluckman and Hanson 2006b.)

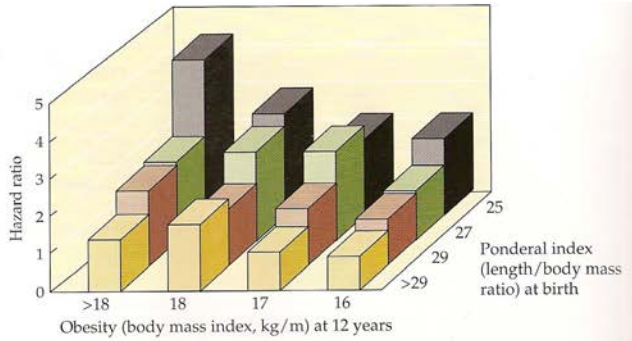


FIGURE 7.10 Data showing that the risk of coronary heart disease is increased by both small birth size and greater fatness in childhood. The graph shows that these conditions interact such that the highest risk of heart disease is in those people who were born at low birth weight (i.e., in conditions that may have caused a thrifty phenotype) but subsequently experienced ample nutrition. (After Eriksson et al. 2001.)

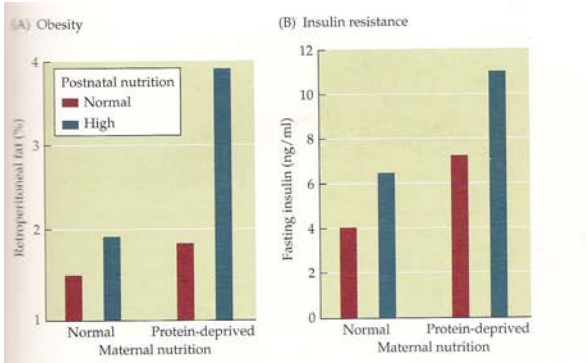


FIGURE 7.11 Experimental evidence for the fetal origin of adult disease in rats. Rat fetuses were exposed to either normal or protein-deficient maternal diets. After birth, the pups were given either normal or high nutrition. They were then measured for (A) obesity (percent fatty tissue in the abdominal area between the peritoneal cavity and the skin) and (B) insulin resistance (as measured by the amount of insulin in their blood after fasting). Obesity and insulin resistance were greater in the pups whose mothers were fed a protein-deficient diet. High nutrition after birth heightened these differences. (After Vickers et al. 2000.)

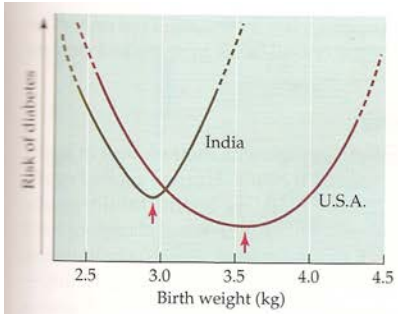
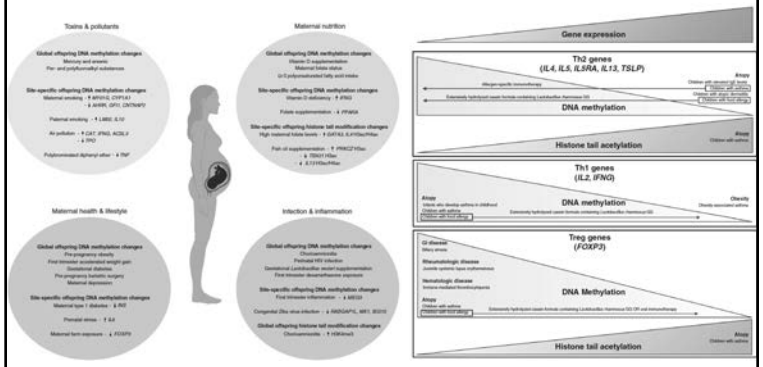
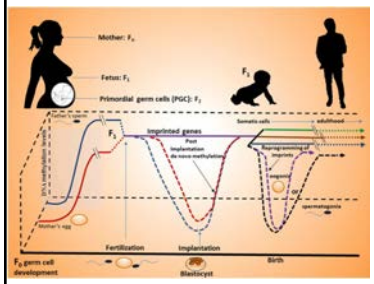


FIGURE 7.13 The risk of adult-onset diabetes is related to birth weight in both India and the United States. The U-shaped curve indicates that the risk for this disease increases at both very high and very low birth weights. However, the optimum birth weight is different for the two populations (arrows), suggesting that developmental plasticity may have resulted in adaptation to distinct adult nutritional milieus. (After Gluckman and Hansen 2005.)

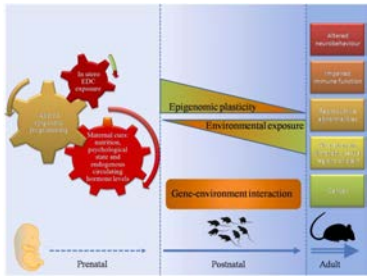
Epigenetic regulation of pediatric and neonatal immune responses.
Bermick J, Schaller M.
Pediatr Res. 2022 Jan;91(2):297-327.



In utero exposure to endocrine-disrupting chemicals, maternal factors and alterations in the epigenetic landscape underlying later-life health effects.
Lite C, Raja GL, Juliet M, Sridhar VV, Subhashree KD, Kumar P, Chakraborty P, Arockiaraj J.
Environ Toxicol Pharmacol. 2022 Jan;89:103779.



Global DNA methylation dynamics.



Susceptibility of epigenetic programming process and adult diseases.

Environmental Epigenetics (Cell Culture Effects)

IVC conditions

Short term consequences

Reduced blastocyst cell number
Altered gene expression patterns
Altered metabolism
Reduced viability

Long term programming effects

Altered fetal/postnatal growth and organ sizing (altered heart structure)
Elevated lung/serum ACE activity
Altered nephron number
Elevated postnatal blood pressure
Behavioral changes
Genome-wide changes in epigenetic status

Long term outcome

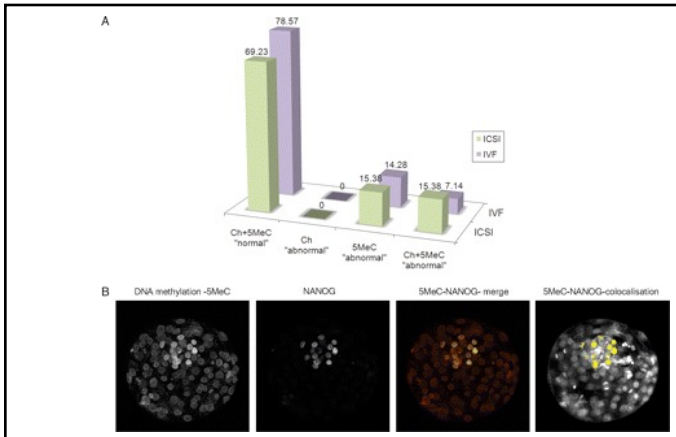
Obesity
Cardiovascular disease and hypertension
Transgenerational inheritage

Long-term programming of postnatal growth and physiology can be induced irreversibly during the preimplantation period of development by adverse preconceptional environment in vitro (for example suboptimal in vitro culture).

Evaluation of epigenetic marks in human embryos derived from IVF and ICSI.

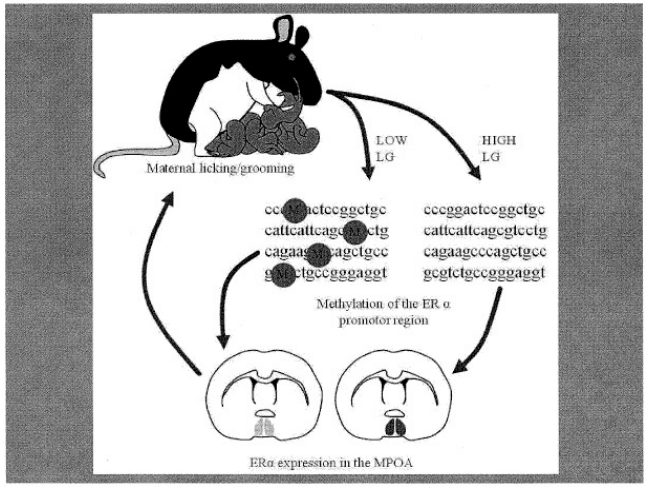
Santos F, Hyslop L, Stojkovic P, Leary C, Murdoch A, Reik W, Stojkovic M, Herbert M, Dean W.

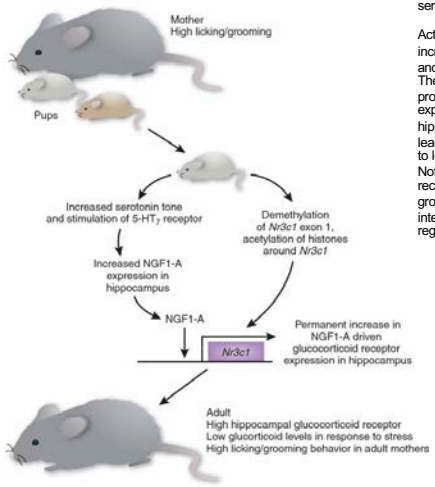
Hum Reprod. 2010 Sep;25(9):2387-95.



Epigenetic lineage asymmetry is conserved in human embryos. (A) Assessment of epigenotypes in blastocysts arising from ART procedures. Blastocysts generated by IVF (n = 14) or ICSI (n = 13) were stained and are portrayed in the discontinuous plot from double normal to double abnormal scores. In contrast to other embryo stages obtained throughout the study, blastocysts showed an overwhelmingly significant positive correlation with normal epigenotype scores (Table III, $P < 0.001$). (B) High morphology score expanded blastocysts were labelled for a lineage identification marker, NANOG, followed by staining for 5MeC. ICM cells were consistently hypermethylated and positive for NANOG. Co-localisation software, Image J, was used to analyse potential association between the epigenetic mark and the pluripotency factor. Bright yellow cells identified most, but not all, of the cells of the ICM, highlighting the intrinsic heterogeneity associated with this compartment. In the expanded blastocyst NANOG is highly specific for the ICM and hence is justifiably used as a lineage marker.

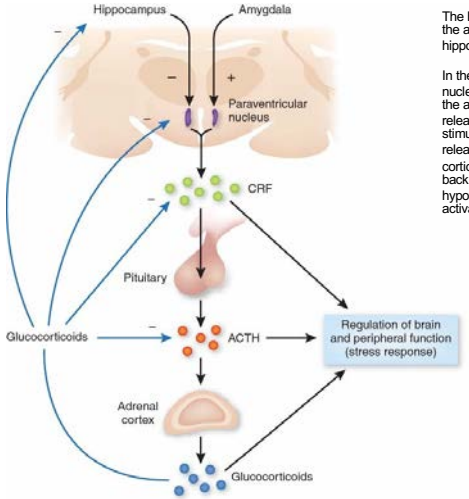
Environmental Epigenetics (Early Life History Brain Effects)





High licking and grooming of rat pups increases serotonin tone in the brain.

Acting via 5-HT7 receptors and cyclic AMP, this increases expression of NGF1-A, which binds to and activates the promoter of the Nr3c1 gene. The pups also show demethylation of the Nr3c1 promoter, which facilitates long-term gene expression. The result is high levels of hippocampal glucocorticoid receptor expression, leading to enhanced feedback inhibition and thus to low cortisol levels in response to stress. Notably, females with high levels of glucocorticoid receptor expression show high licking and grooming behaviors on their offspring, and thus intergenerational transmission of epigenetic regulation of the Nr3c1 gene.



The HPA axis is under the excitatory control of the amygdala and inhibitory control of the hippocampus.

In the hypothalamus, the paraventricular nucleus releases CRF, which is transported to the anterior pituitary, where it causes the release of ACTH into the blood stream. ACTH stimulates the adrenal cortex to synthesize and release the glucocorticoids cortisol (humans) or corticosterone (rodents). Glucocorticoids feed back at the level of the hippocampus, hypothalamus and pituitary to dampen excess activation of the HPA axis.

Lasting epigenetic influence of early-life adversity on the BDNF gene.

Roth TL, Lubin FD, Funk AJ, Sweatt JD.

Biol Psychiatry. 2009 May 1;65(9):760-9.

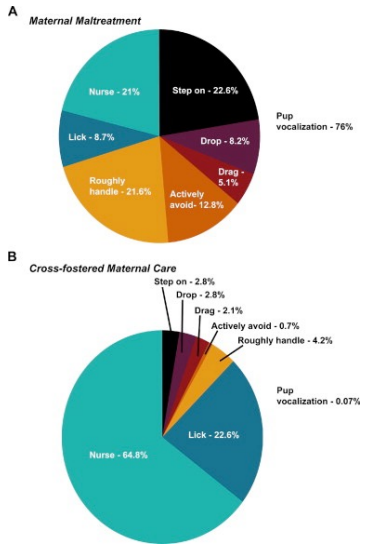


Figure 1. Infants experienced an adverse caregiving environment. (A) Qualitative assessment of the percent occurrence of pup-directed behaviors in the maltreatment condition indicates that pups experienced predominately abusive behaviors, which resulted in considerable audible pup vocalization. (B) In sharp contrast, pups experienced significant amounts of normal maternal care behaviors in the cross-fostered maternal care condition. Statistical analyses of the maternal behaviors (abusive vs. normal care) are provided in Supplement 3.

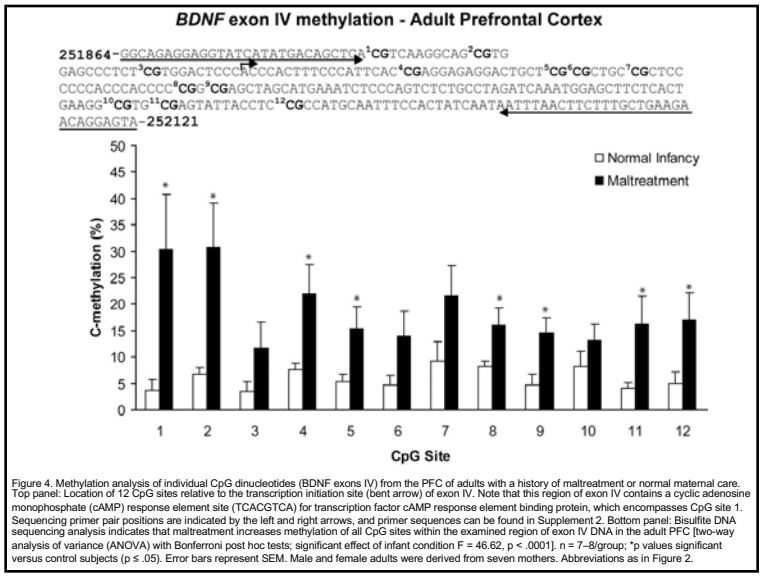
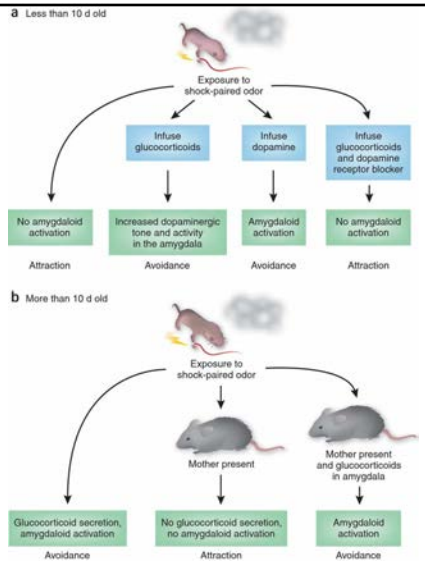


Figure 4. Methylation analysis of individual CpG dinucleotides (BDNF exons IV) from the PFC of adults with a history of maltreatment or normal maternal care. Top panel: Location of 12 CpG sites relative to the transcription initiation site (bent arrow) of exon IV. Note that this region of exon IV contains a cyclic adenosine monophosphate (cAMP) response element site (TCACGTCA) for transcription factor cAMP response element binding protein, which encompasses CpG site 1. Sequencing primer pair positions are indicated by the left and right arrows, and primer sequences can be found in Supplement 2. Bottom panel: Bisulfite DNA sequencing analysis indicates that maltreatment increases methylation of all CpG sites within the examined region of exon IV DNA in the adult PFC [two-way analysis of variance (ANOVA) with Bonferroni post hoc tests; significant effect of infant condition $F = 46.62$, $p < .0001$]. $n = 7–8$/group; *p values significant versus control subjects ($p \leq .05$). Error bars represent SEM. Male and female adults were derived from seven mothers. Abbreviations as in Figure 2.



Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse.

McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonté B, Szyf M, Turecki G, Meaney MJ.

Nat Neurosci. 2009 Mar;12(3):342-8.

Table 1 Demographic characteristics and psychiatric diagnoses

	Abused suicide	Nonabused suicide	Control
Male/female	12/0	12/0	12/0
Age (years)	34.2 ± 10	33.8 ± 11	35.8 ± 12
PMI (h)	24.6 ± 5.8	39.0 ± 25.7	23.5 ± 6.0
pH	6.3 ± 0.24	6.5 ± 0.29	6.5 ± 0.22
Childhood abuse/neglect	12/0 (100%)	0/12 (0%)	0/12 (0%)
Mood disorder	8/12 (67%)	8/12 (67%)	0/12 (0%)
Alcohol/drug abuse disorder	9/12 (75%)	6/12 (50%)	5/12 (42%)

Data are presented as mean ± s.d.

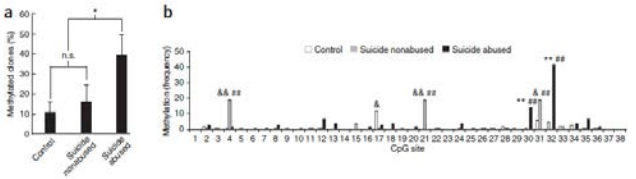
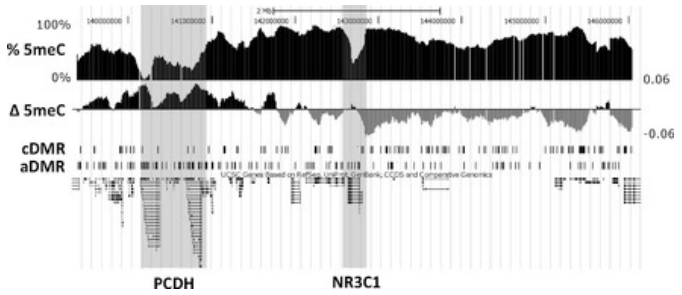
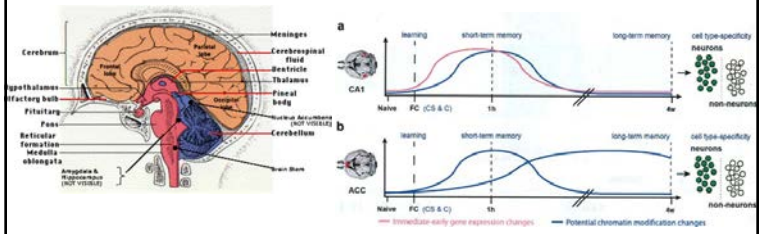


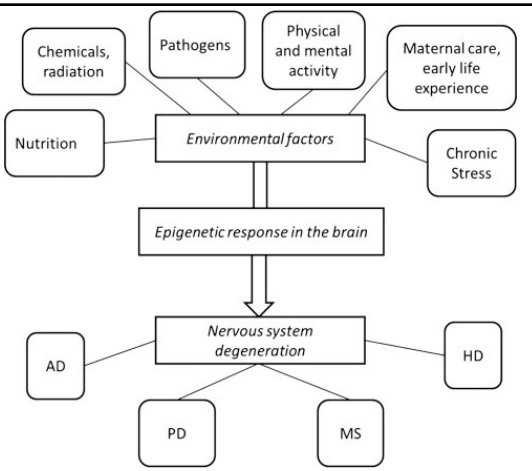
Figure 2 Methylation of the *NR3C1* promoter in the hippocampus. Twenty clones were sequenced for each subject for methylation mapping. (**a**) Mean ± s.e.m. percentage of methylated clones for suicide victims with a history of childhood abuse ($n = 12$), suicide victims without a history of childhood abuse ($n = 12$) and controls ($n = 12$). The methylation percentage was calculated as the number of clones with at least one methylated CpG site divided by the total number of clones (* indicates $P \leq 0.05$; n.s. indicates not statistically significant). (**b**) Methylation of the *NR3C1* promoter region, showing the frequency of methylation observed at each CpG site for suicide victims with a history of childhood abuse, suicide victims with no history of childhood abuse and control subjects (*$P < 0.05$, **$P < 0.001$, abused suicides versus controls; $^{\&}P < 0.05$, $^{\&\&}P < 0.001$, non-abused suicides versus controls; $^{\#}P < 0.05$, $^{\#\#}P < 0.001$, abused suicides versus non-abused suicides; Bonferroni *post hoc* comparisons).

Suderman M, et al. (2012) Conserved epigenetic sensitivity to early life experience in the rat and human hippocampus. Proc Natl Acad Sci U S A. 16;109 Suppl 2:17266-72

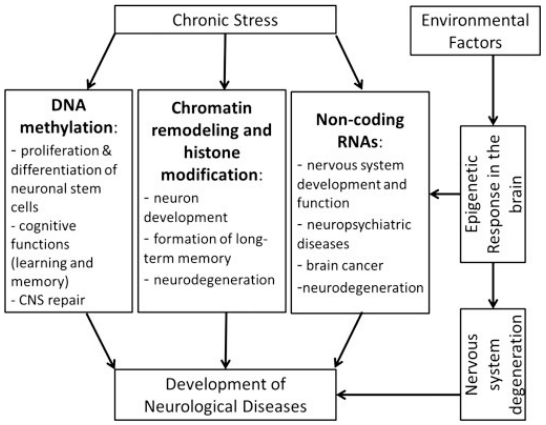


Effect of stress on human biology: Epigenetics, adaptation, inheritance, and social significance.
J Cell Physiol. 2018 Mar;233(3):1975-1984.
Denhardt DT.



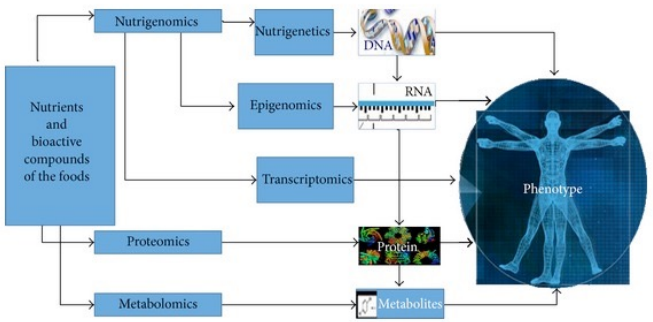


Role of epigenetic regulation as a central regulatory interface that translates the relationship between brain and behavior into patterns of gene expression. Different environmental factors such as nutrition, physical and mental activity, maternal care and early life experience, exposure to chronic stress, chemicals and radiation, and pathogens can alter the epigenetic pattern in the brain. These processes in turn may either delay or accelerate neurodegenerative processes in the nervous system and contribute to the onset and progression of neurological conditions, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and multiple sclerosis (MS).



Stress might be a main contributing factor to enhanced risk and accelerated progression of neurodegenerative diseases. The intermediate link between stress and neurodegeneration represent epigenetic components, such as DNA methylation, chromatin remodeling and histone modification, and non-coding RNAs in the nervous system.

Environmental Epigenetics (Nutrition Effects)



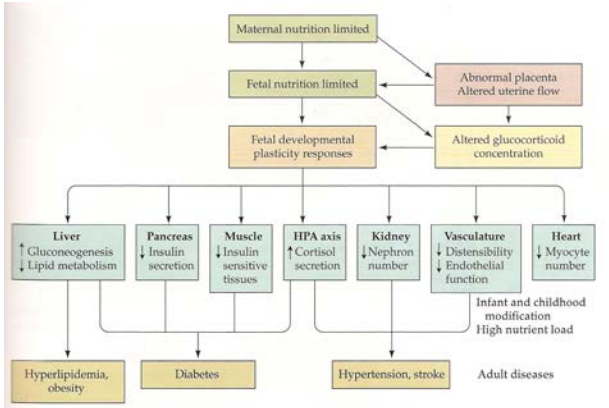
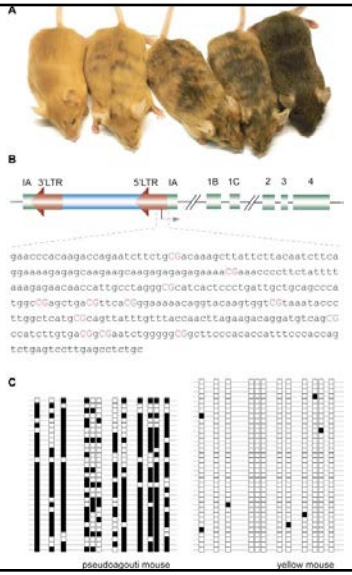


FIGURE 7.6 The thrifty phenotype hypothesis. Altered maternal nutrition leads to a cascade of consequences mediated by the phenotypic plasticity of the fetal organs. The combination of biochemical and anatomical changes in these organs produces a phenotype that stores excess energy as fats and sacrifices kidney growth for brain growth. This phenotype is beneficial under environments of low nutrition, but high nutrition during infancy and/or childhood can result in adult obesity, diabetes, and hypertension. (After De Boo and Harding 2006.)

Table 3. Effect of maternal dietary modifications on imprinted gene, IGF2

Species	Modification during development	Time winnow for the modification	Observations in offspring	Reference
Human	Famine (Dutch Famine cohort)	Periconceptional period	Decrease in methylation of CpG dinucleotides in the IGF2 nearly 60 years after	[35]
Human	Supplementary folic acid use	Periconceptional period	Higher methylation of the IGF2 differentially methylated region (DMR)	[62]
Rat	Maternal low protein diet (8% vs 20 %) and high fat diet (45% vs 10%) after weaning	2 weeks prior to mating – gestation – lactation	Increase in adipose tissue Igf2 mRNAs by the low protein prenatal diet	[38]
Rat	Maternal low protein diet (9% vs 19% control)	Preimplantation period	Decrease in H19 imprinted gene expression in male blastocysts; reduction in H19 and Igf2 expression in male fetal liver at day 20 of gestation	[36]
Mouse	Methyl deficiency (methionine, choline, folic acid and vitamin B12)	60-day post-weaning	Loss of imprinting of Igf2	[37]



Phenotypic variation in A^{vy} mice, A^{vy} allele structure, and complex CpG methylation at the IAP.

A. Phenotypes of isogenic A^{vy} littermates range from pure yellow and obese (left) through mottled yellow/agouti to lean fully agouti, called pseudoagouti (right). **B.** Schematic of the A^{vy} locus, with the sequence of the amplified region, which includes portions of the 5′LTR and pseudoexon 1A (−240 to +92 relative to the cryptic promoter, which is marked by an arrow). The start point of Avy transcription [11] is marked by an arrow. CpG dinucleotides are displayed in red. **C.** Representative bisulphite allelic sequencing profiles of individual alleles from yellow and pseudoagouti mice. Each single row represents a single allele, and each box a CpG (white: unmethylated; black: methylated).

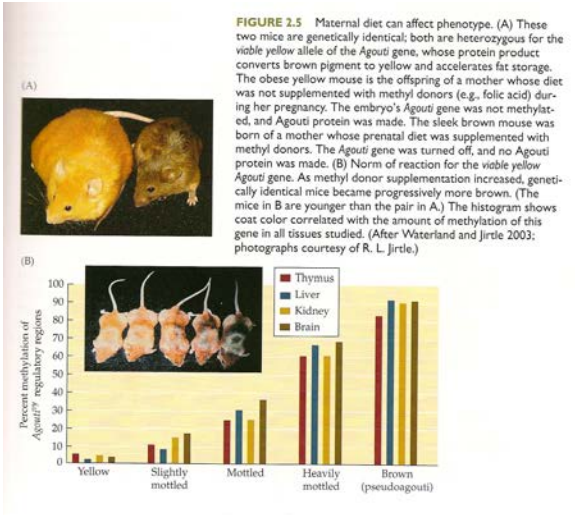
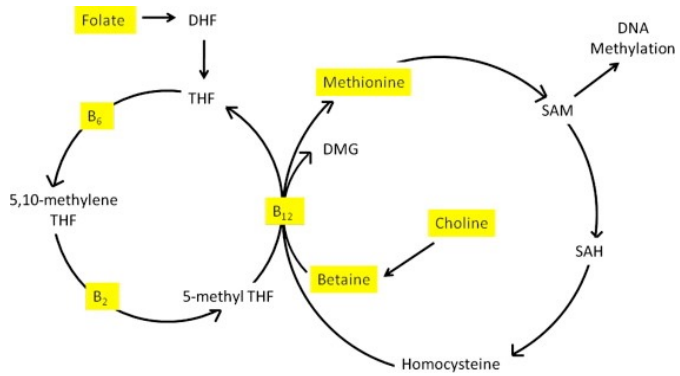
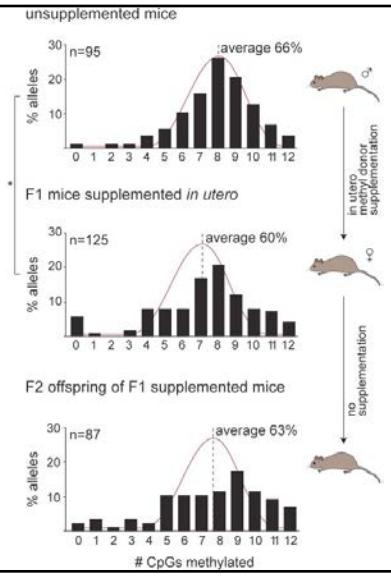
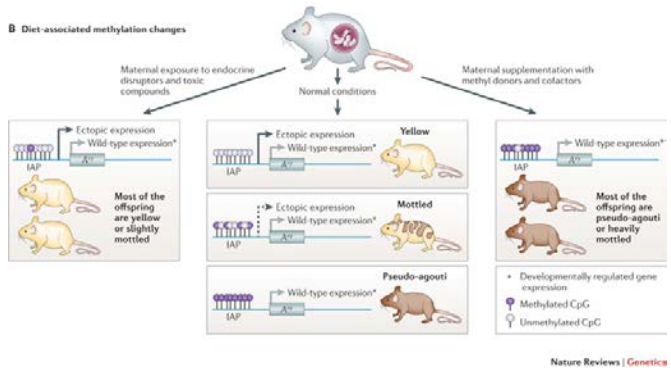
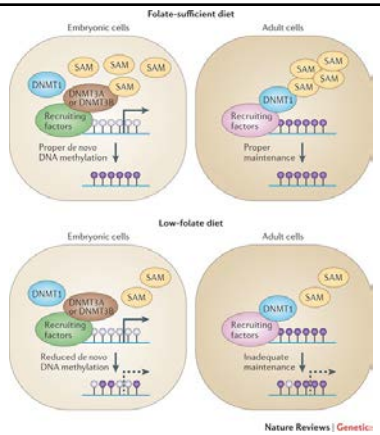


FIGURE 2.5 Maternal diet can affect phenotype. (A) These two mice are genetically identical; both are heterozygous for the *viable yellow* allele of the *Agouti* gene, whose protein product converts brown pigment to yellow and accelerates fat storage. The obese yellow mouse is the offspring of a mother whose diet was not supplemented with methyl donors (e.g., folic acid) during her pregnancy. The embryo's *Agouti* gene was not methylated, and Agouti protein was made. The sleek brown mouse was born of a mother whose prenatal diet was supplemented with methyl donors. The *Agouti* gene was turned off, and no Agouti protein was made. (B) Norm of reaction for the *viable yellow Agouti* gene. As methyl donor supplementation increased, genetically identical mice became progressively more brown. (The mice in B are younger than the pair in A.) The histogram shows coat color correlated with the amount of methylation of this gene in all tissues studied. (After Waterland and Jirtle 2003; photographs courtesy of R. L. Jirtle.)









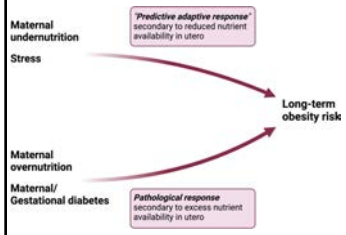
Epigenetic transitions play crucial roles in development and in the differentiation of stem cells and primordial germ cells. Concordantly, the regulating enzymes are generally highly expressed in these pluripotent cells6. For example, the de novo methyltransferases, DNMT3A and DNMT3B, are highly expressed in the early embryonic cells in which de novo methylation is acquired. Low amounts of external methyl donor groups from dietary sources can reduce the concentrations of the universal methyl donor, S-adenosylmethionine (SAM), and can readily affect de novo DNA methylation. Also, aberrant gains of methylation may occur in early embryonic cells owing to other external triggers. In adult cells, the maintenance of DNA methylation is performed mainly by the maintenance methyltransferase, DNMT1, in a process that seems less sensitive to diet-induced changes in the abundance of methyl donors.

Increased cancer incidence in Holocaust survivors and the implications for survivors of other extreme events.
Expert Rev Anticancer Ther. 2018 Nov;18(11):1059-1062.
Keinan-Boker L.

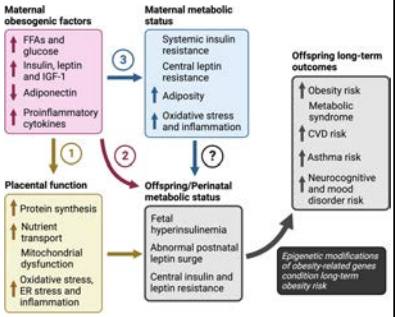
3. Conclusions

Studies show unequivocally that restricted diet in lab animals reduces cancer risk. Observational studies in non-Jewish European populations yielded mixed results which may be attributed to differing study types, definitions of exposure, the selection of control subjects, the nature of the exposure etc. In contrast, most studies of Holocaust survivors clearly indicated an inverse effect despite differing methodologies. Although these findings need consolidation, there are grounds for believing that exposure to hunger and stress under extreme situations may cause a cascade of epigenetic, hormonal, and biological changes that eventually modify cancer risk. Thus, exposed individuals should be regarded as a high risk group for cancer. Holocaust survivors are one example of such a group; these conclusions may be generalized to many populations around the globe, including, for example, the survivors of the civil war in Syria.

The metabolic conditioning of obesity: A review of the pathogenesis of obesity and the epigenetic pathways that "program" obesity from conception.
Rajamoorthi A, LeDuc CA, Thaker VV.
Front Endocrinol (Lausanne). 2022 Oct 18;13:1032491.

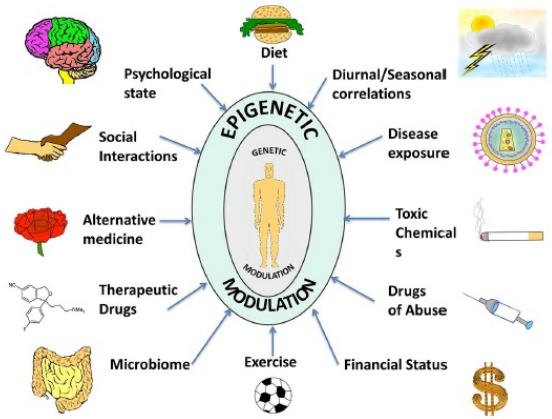


Differential pathways to obesity in response to maternal undernutrition vs overnutrition.



Pathways to obesity in children in response to maternal overnutrition.

Environmental Epigenetics (Toxicant Exposures)



Environmental Deflection: The Impact of Toxicant Exposures on the Aging Epigenome.
Kochmanski J, Montrose L, Goodrich JM, Dolinoy DC.
Toxicol Sci. 2017 [Epub ahead of print]

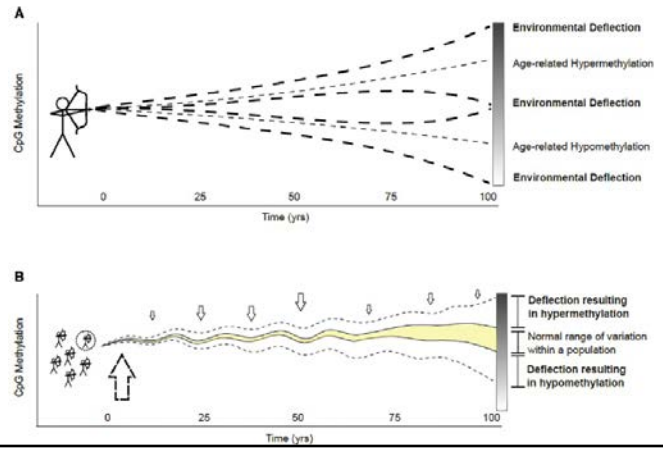
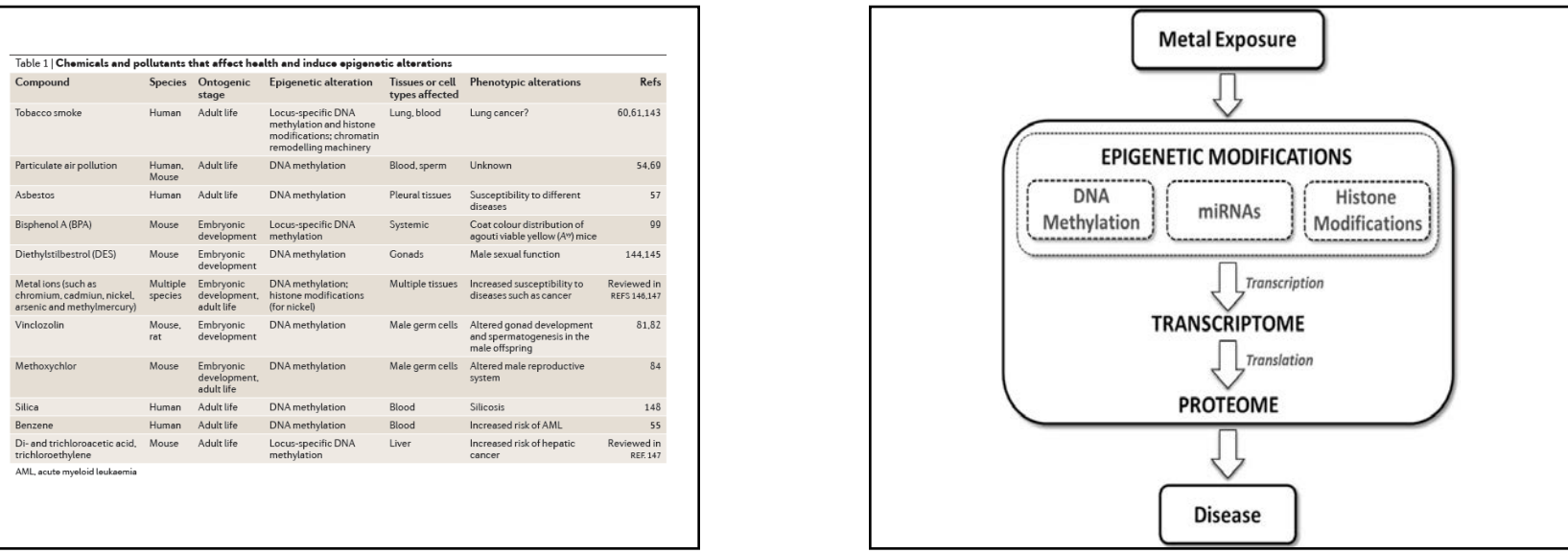


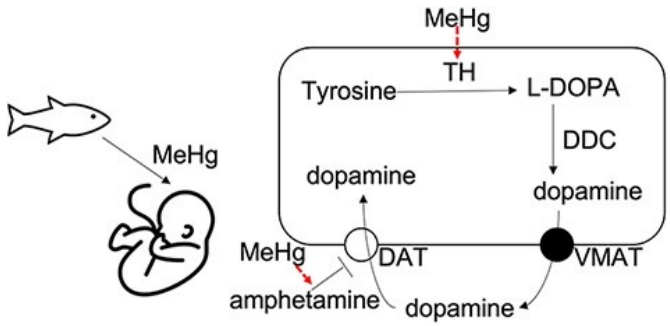
Table 1 | **Chemicals and pollutants that affect health and induce epigenetic alterations**

Compound	Species	Ontogenic stage	Epigenetic alteration	Tissues or cell types affected	Phenotypic alterations	Refs
Tobacco smoke	Human	Adult life	Locus-specific DNA methylation and histone modifications; chromatin remodelling machinery	Lung, blood	Lung cancer?	60,61,143
Particulate air pollution	Human, Mouse	Adult life	DNA methylation	Blood, sperm	Unknown	54,60
Asbestos	Human	Adult life	DNA methylation	Pleural tissues	Susceptibility to different diseases	57
Bisphenol A (BPA)	Mouse	Embryonic development	Locus-specific DNA methylation	Systemic	Coat colour distribution of agouti viable yellow (A^{vy}) mice	99
Diethylstilbestrol (DES)	Mouse	Embryonic development	DNA methylation	Gonads	Male sexual function	144,145
Metal ions (such as chromium, cadmium, nickel, arsenic and methylmercury)	Multiple species	Embryonic development, adult life	DNA methylation; histone modifications (for nickel)	Multiple tissues	Increased susceptibility to diseases such as cancer	Reviewed in REFS 146,147
Vinclozolin	Mouse, rat	Embryonic development	DNA methylation	Male germ cells	Altered gonad development and spermatogenesis in the male offspring	81,82
Methoxychlor	Mouse	Embryonic development, adult life	DNA methylation	Male germ cells	Altered male reproductive system	84
Silica	Human	Adult life	DNA methylation	Blood	Silicosis	148
Benzene	Human	Adult life	DNA methylation	Blood	Increased risk of AML	55
Di- and trichloroacetic acid, trichloroethylene	Mouse	Adult life	Locus-specific DNA methylation	Liver	Increased risk of hepatic cancer	Reviewed in REF.147

AML, acute myeloid leukaemia



Developmental exposure to methylmercury and ADHD, a literature review of epigenetic studies.
Ke T, Tinkov AA, Skalny AV, Bowman AB, Rocha JBT, Santamaria A, Aschner M.
Environ Epigenet. 2021 Nov 22;7(1):dvab014.



Potential impacts of developmental MeHg exposures on dopamine neurotransmission. The developing brain of fetus is susceptible to environmental exposure to neurotoxins. The primary pathway for dopamine synthesis involves several enzymes including TH and DDC. For the dopamine neurotransmission, MeHg exposure can alter the epigenetic regulation of the TH gene and potentiate the effect of dopamine neurotransmission agonists such as amphetamine [55–58]. TH, tyrosine hydroxylase; L-DOPA, L-3,4-dihydroxyphenylalanine; DDC, DOPA decarboxylase; VMAT, vesicular monoamine transporter 2; DAT, dopamine transporter

Table 3. Summary of studies exploring epigenetic effects of mercury

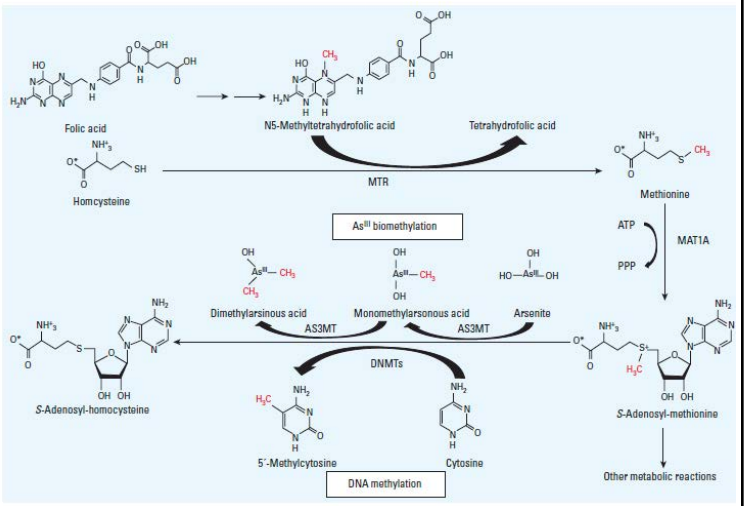
Species	Tissue/cell type	Chemical	Effect	Reference
Polar bear	Brainstem	MeHg	Reduced global DNA methylation in male bears but not in female bears	[67]
Mink	Occipital cortex	MeHg	Reduced global DNA methylation, reduced DNMT activity	[68]
Chicken	Cerebrum	MeHg	No effect on global DNA methylation or DNMT activity	[68]
Yellow perch	Telencephalon	MeHg	No effect on global DNA methylation	[68]
Earthworm	Whole	Hg	Reduced global DNA methylation	[71]
Mouse	Brain hippocampus	MeHg	Suppression of the *Bdnf* promoter via hypermethylation, increased histone H3K27 trimethylation, and decreased histone H3 acetylation	[73]
Mouse	Embryonic stem cells	Hg	Reversible alterations to heterochromatin Hypermethylation of *Rnd2* gene	[75]
Mouse	Embryonic stem cells	Hg	Reduction of total histone protein levels and H3K27 monomethylation	[74]
Rat	Liver	MeHg	Reduced *Dnmt1* and *Dnmt3b* mRNA expression, decreased CpG methylation at *Cdkn2a* promoter, and no effect on global DNA methylation or SAM abundance	[70]
Rat	Primary cultures of embryonic cortical neural stem cells	MeHg	Decreased global DNA methylation, and downregulation of *Dnmt1b* mRNA	[69]
Human	Blood	Hg	Hypermethylation of the *GSTM1/5* promoter	[76]
Human	Buccal cells	MeHg	Hypomethylation of *SEPP1* gene among males	[77]

MeHg = methylmercury; DNMT = DNA methyltransferase; SAM = S-adenosylmethionine.

Table 2 Effects of environmental chemicals on histones

Exposure	↑/↓*	Modification	Type	Tissue	References
Nickel	↓	Acetylation	*In vitro*	Liver, brain	Ke *et al.* [38]
	↑	H3K9 dimethylation			
	↑	H2A and H2B monoubiquitination			
	↓	H4K12 acetylation	*In vitro*	Yeast cells	Broday *et al.* [39]
	↓	H4K4 acetylation	*In vitro*	Mammalian cells	
	↑	H3K9 monomethylation and dimethylation	*In vitro*	G12 cell line	Chen *et al.* [40]
	↓	Acetylation of histone H2B	*In vitro*	HAE and NRK cell lines	Golebiowski and Kasprzak [41]
	↓↑	H2B ubiquitination	*In vitro*	HAEo and HPL1D cell lines	Karaczyn *et al.* [42]
Arsenic	↑	H3K9 dimethylation	*In vitro*	A549 cell line	Zhou *et al.* [43]
	↓	H3K27 trimethylation			
	↑	H3K4 trimethylation			

HAE, human airway epithelial; NRK, normal rat kidney.
* Increase (↑) or decrease (↓) in histone modification.



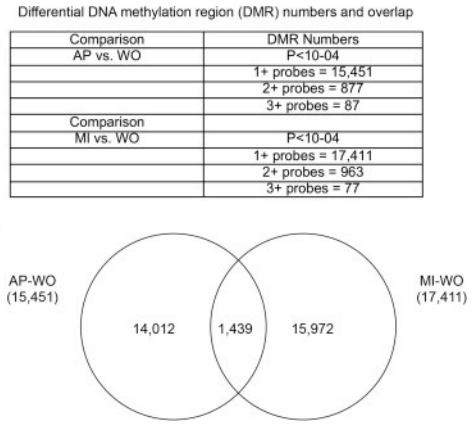
Epigenetic programming alterations in alligators from environmentally contaminated lakes.

Gen Comp Endocrinol. 2016 Nov 1;238:4-12.

Guillette LJ Jr, Parrott BB, Nilsson E, Haque MM, Skinner MK.

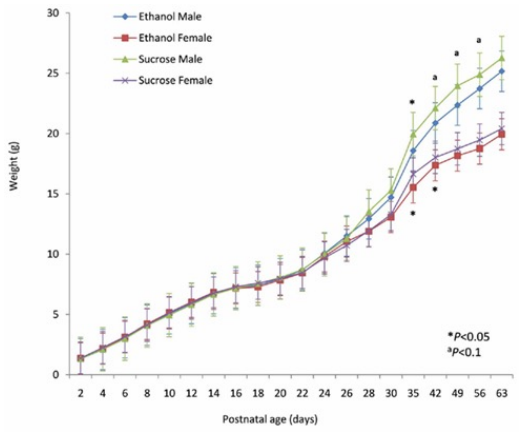
Representative examples of endocrine actions of various environmental contaminants on wildlife and documented health perturbations. American alligators from three sampling sites in Florida, USA: AP, Lake Apopka; MI, Merritt Island National Wildlife Refuge; and WO, Lake Woodruff National Wildlife Refuge. Endocrine activity associated with specific contaminants are presented. Health effects previously documented (Guillette, 2000; Rosai et al., 2014) are listed.

Site	Associated contaminants	Endocrine activity	Health perturbations in alligators
Lake Apopka	p,p′-DDE	Xenoestrogen (Warembourg et al., 2016) Antiandrogen (Wilson et al., 2008)	Altered sex steroid hormone concentrations (Guillette et al., 1994, 1996; Moore et al., 2010)
	p,p′-DDD	Xenoestrogen (Zhuang et al., 2012)	Reduced penis size (Guillette et al., 1994)
	trans-nonachlor	Xenoestrogen (Kelce et al., 2010)	Abated response to gonadotropin stimulation (Moore et al., 2010, 2012)
	Dieldrin	Altered hormone metabolism (Fowler et al., 2007) Xenoestrogen (Soto et al., 1994)	Increased presence of polyovular follicles (Guillette et al., 1994)
	Mirex	Xenoestrogen (Fenin et al., 2014; Warner, 1987)	Decreased robustness of sexually dimorphic gene expression (Milnes et al. 2008)
	Toxaphene	Xenoestrogen (Hodges et al., 2000; Scippo et al., 2004)	Decreased hatch rate (Milnes et al., 2008; Woodward et al., 1993)
Merritt Island	Elevated Li, Fe, Ni, Sr, Sb, Pb, Bi, V, As, S	Thyroid hormone changes (Aperniti and Catalani, 2011; Lazarus, 2009) HPG axis abnormalities (Rossi et al., 2016) Metalloestrogen (Aperniti and Catalani, 2011; Aquino et al., 2012; Darbre, 2006; Faroon, 2015; Gosse et al., 2014; Martin-Diaz et al., 2008) Decreased testosterone (Hatton, 2005; Kariyawasa et al., 2015; Rooker et al., 2013)	Reduced length (Trillanes et al., 2014) Decreased hatch rate (Lance et al., 2006)
Lake Woodruff	Reference site	None	None



DMR numbers and overlap. (A) The comparison AP vs. WO and MI vs. WO with $p < 10^{-4}$ for single oligo probe (1+), two adjacent oligo probes (2+) and $\geqslant 3$ adjacent oligo probes (3+) detection. (B) Venn diagram overlap for single (1+) probe overlap. The DMR had to have overlapping sequence at the same genomic locations to be considered overlapped.

Knezovich JG, Ramsay M. (2012) The effect of preconception paternal alcohol exposure on epigenetic remodeling of the h19 and rasgrf1 imprinting control regions in mouse offspring. Front Genet; 3:10.



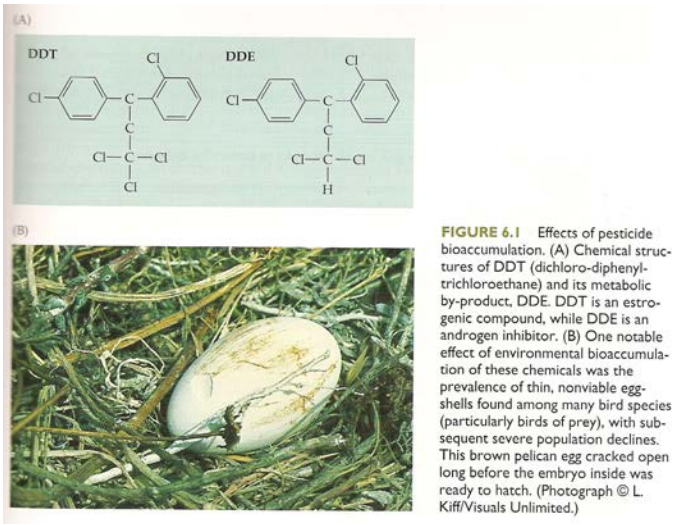
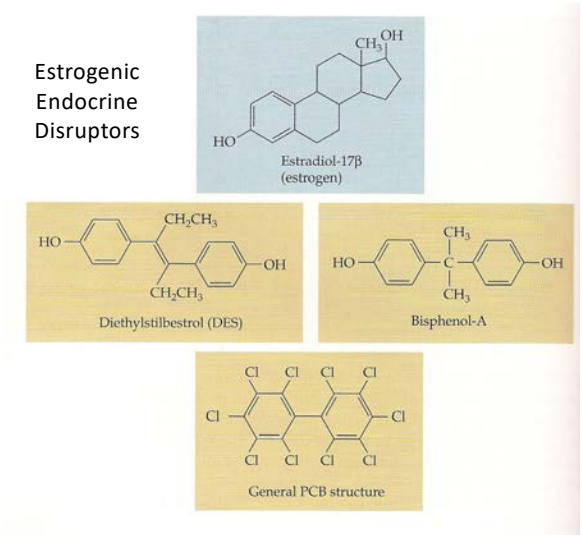
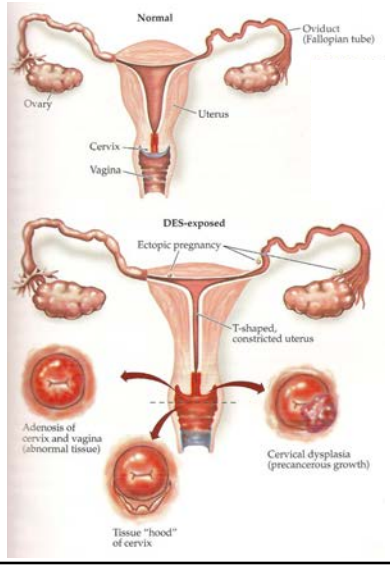
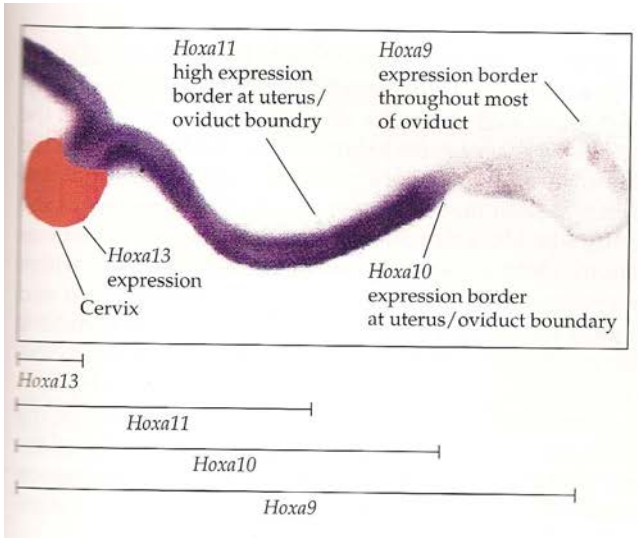


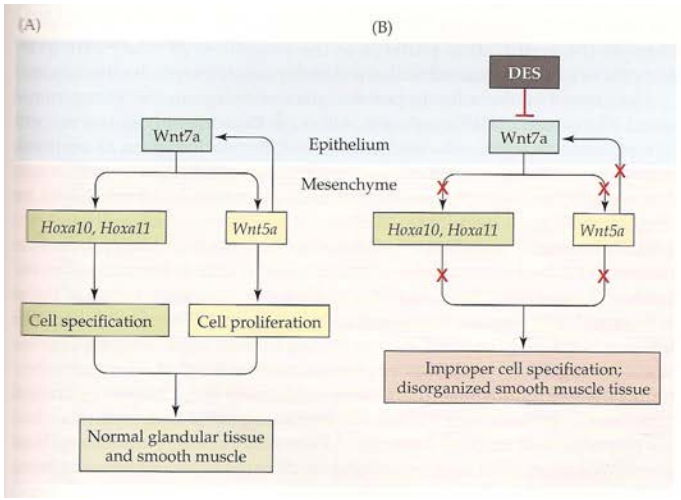
FIGURE 6.1 Effects of pesticide bioaccumulation. (A) Chemical structures of DDT (dichloro-diphenyl-trichloroethane) and its metabolic by-product, DDE. DDT is an estrogenic compound, while DDE is an androgen inhibitor. (B) One notable effect of environmental bioaccumulation of these chemicals was the prevalence of thin, nonviable eggshells found among many bird species (particularly birds of prey), with subsequent severe population declines. This brown pelican egg cracked open long before the embryo inside was ready to hatch. (Photograph © L. Kiff/Visuals Unlimited.)

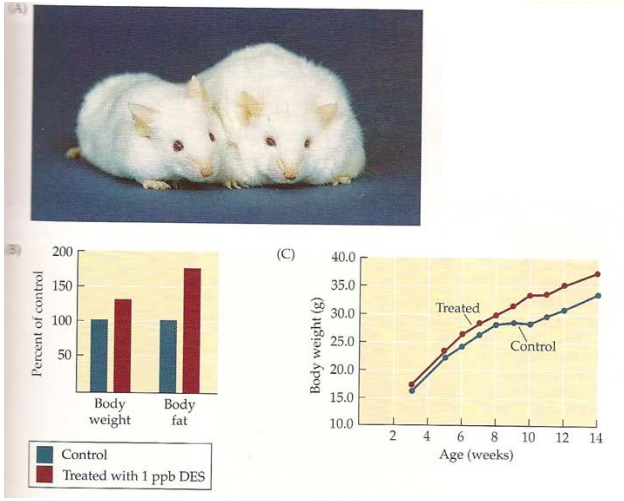
Estrogenic Endocrine Disruptors







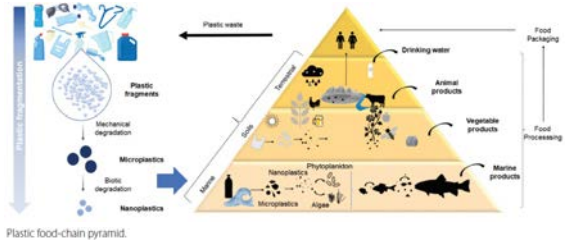




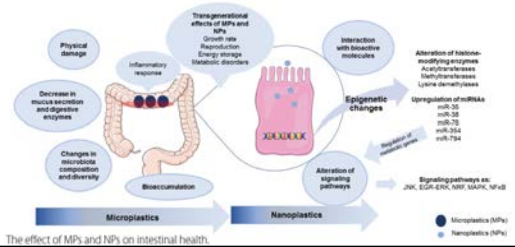
Untoward Effects of Micro- and Nanoplastics: An Expert Review of Their Biological Impact and Epigenetic Effects.

López de Las Hazas MC, Boughanem H, Dávalos A.

Adv Nutr. 2022 Aug 1;13(4):1310-1323.



Plastic food-chain pyramid.



The effect of MPs and NPs on intestinal health.

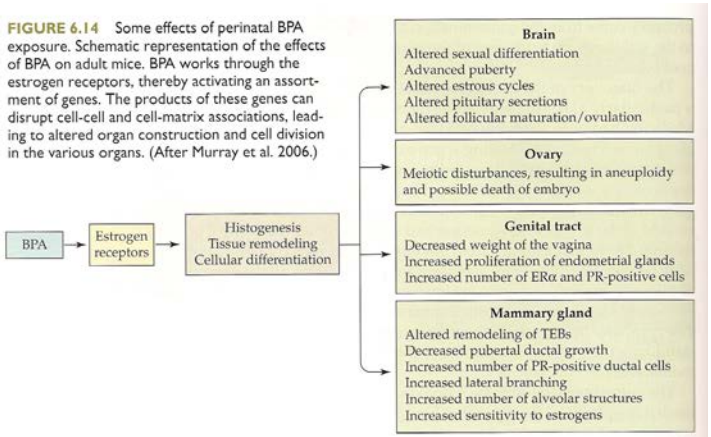
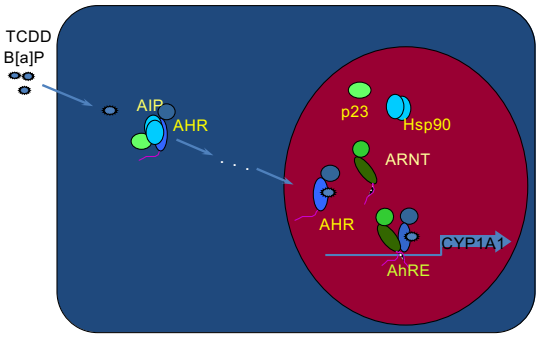


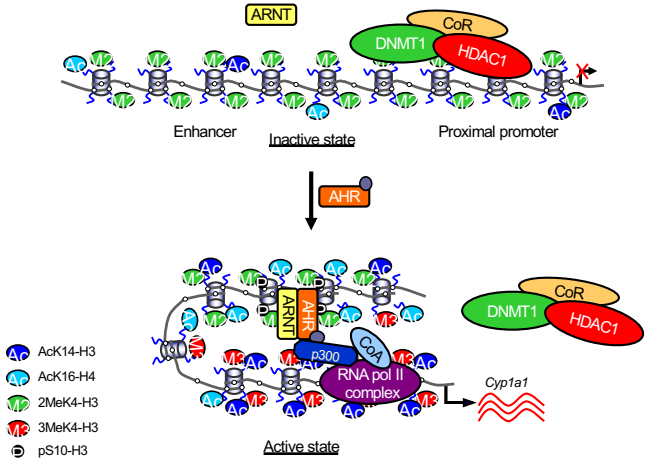
FIGURE 6.14 Some effects of perinatal BPA exposure. Schematic representation of the effects of BPA on adult mice. BPA works through the estrogen receptors, thereby activating an assortment of genes. The products of these genes can disrupt cell-cell and cell-matrix associations, leading to altered organ construction and cell division in the various organs. (After Murray et al. 2006.)

Ah Receptor-Dependent Target Gene Expression

(*simplified view*)



Epigenetic activation of the *Cyp1a1* promoter

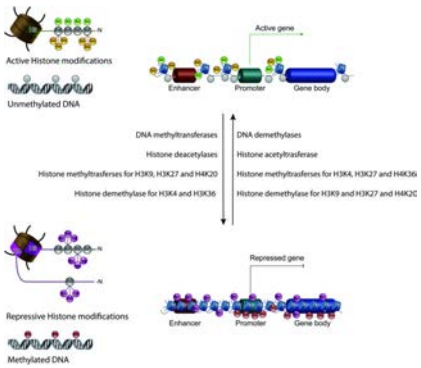


Stoccoro A, Karlsson HL, Coppedè F, Migliore L. (2012) Epigenetic effects of nano-sized materials. Toxicology. 2012 Dec 10. doi: 10.1016/j.tox.2012.12.002. [Epub ahead of print]

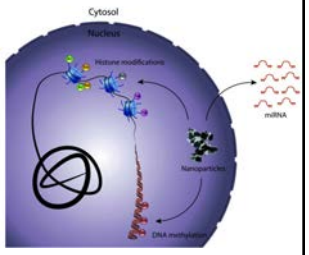
Table 1
Studies on epigenetic effects of fine and ultrafine air pollution particles.

Study model	Particle	Epigenetic effect	Reference
DNA methylation			
Blood cells (*Normative Aging Study*)	$PM_{2.5}$, black carbon	PM2.5 and black carbon associated with hypomethylation of LINE1	Baccarelli et al. (2009)
Blood cells (*Normative Aging Study*)	$PM_{2.5}$, black carbon	Prolonged exposure to black carbon associated with hypomethylation of LINE1 and Alu	Madrigano et al. (2011)
Blood cells (*Normative Aging Study*)	$PM_{2.5}$, particle number, black carbon	Effect from air pollution (inflammation, coagulation, etc.) was stronger among subjects having higher Alu, but lower LINE-1, tissue factor (F3), or Toll-like receptor 2 (TLR-2) methylation status	Bind et al. (2012)
Blood cells (*Steel plant workers*)	PM_{10}, metals	PM10 associated with lower LINE1 and Alu methylation. iNOS methylation was significantly lower in postexposure blood samples (after 3 working days) compared with baseline	Tarantini et al. (2009)
Buccal cells (*Children's Health Study*)	$PM_{2.5}$	Increased 7-day average PM2.5 exposure was associated with lower iNOS methylation	Salam et al. (2012)
Blood cells	Air pollution, $PM_{2.5}$, PM_{10}	Increased exposure to ambient air pollution was associated with hypermethylation of the Foxp3 locus	Nadeau et al. (2010)
Blood cells (*Steel plant workers*)	PM_{10}, PM_{1}, various metals	Promoter DNA methylation levels of *APC* and *p16* were higher in post-exposure samples compared to the levels in baseline samples. Mean levels of *p53* or *RASSF1A* promoter methylation was decreased	Hou et al. (2011)
C57BL/CBA mice (*Sperm*)	Air pollution particles near steel mill and highway	Sperm DNA was hypermethylated in mice breathing air particles when compared to HEPA-filtered air, and this change persisted following removal from the environmental exposure	Yauk et al. (2008)
BALB/c mice (CD4+ cells)	DEP	Diesel particle exposure resulted in hypermethylation of the IFNG promotor and hypomethylation of *Il4* promoter in CD+ cells	Liu et al. (2008)
Mice and cultured lung cells	$PM_{2.5}$	PM 2.5 led to increase expression of the DNA methyltransferase 1 (DNMT1), and methylation of the *p16* promoter in mice and cells.	Soberanes et al. (2012)
Histone modifications			
Blood cells (*Steel plant workers*)	PM_{10}, PM_{1}, various metals	H3K4me2 and H3K9ac increased in association with years of employment in the steel plant. No clear relation to exposure to total mass of PM10 or PM1 but to inhalable nickel and arsenic.	Cantone et al. (2011)
A549 cell line	PM_{10}	PM10 induced histone H4 acetylation at the *IL8* promoter as well as increased *IL8* expression.	Gilmour et al. (2003)
BEAS-2B cells	DEP	Diesel particle exposure led to increased histone H4 acetylation at the *COX2* promoter as well as increased *COX2* expression.	Cao et al. (2007)
miRNA expression			
Human primary bronchial epithelial cells	DEP	Diesel particle exposure led to changes in miRNA expression: miR-513, miR-494 and miR-923 were up-regulated whereas miR-96 was down-regulated	Jardim et al. (2009)

The Yin and Yang of epigenetics in the field of nanoparticles.
Musolino E, Pagiatakis C, Serio S, Borgese M, Gamberoni F, Gornati R, Bernardini G, Papait R.
Nanoscale Adv. 2022 Jan 10;4(4):979-994.

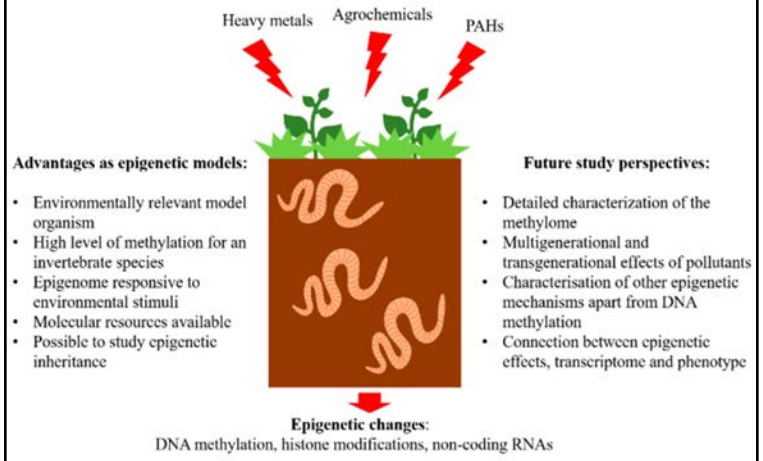


The epigenetic code

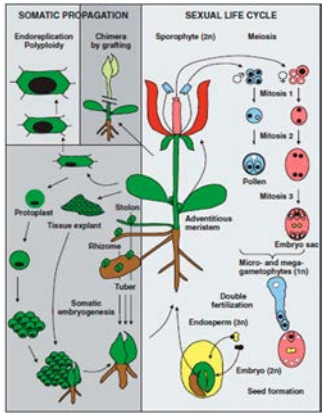


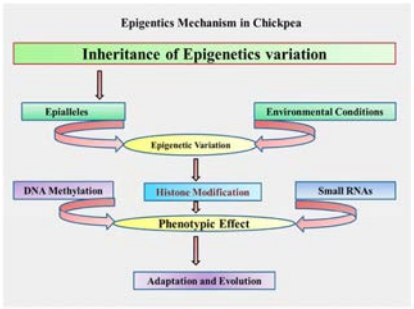
Nanotoxicity at the epigenetic level

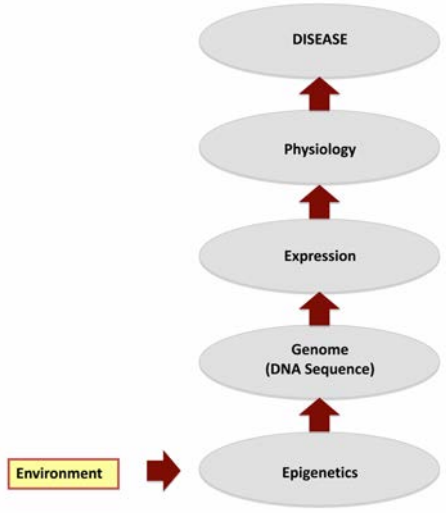
Environmental Epigenetics in Soil Ecosystems: Earthworms as Model Organisms.
Šrut M.
Toxics. 2022 Jul 20;10(7):406.



Epigenomics as Potential Tools for Enhancing Magnitude of Breeding Approaches for Developing Climate Resilient Chickpea.
Chandana BS, Mahto RK, Singh RK, Ford R, Vaghefi N, Gupta SK, Yadav HK, Manohar M, Kumar R.
Front Genet. 2022 Jul 22;13:900253.







"Epigenetics and Systems Biology"

Spring 2023 (Odd Years)
Biol 476/576
Schedule/Lecture Outline –

Week 1	January 10 & 12	Systems Biology (History/ Definitions/ Theory)
Week 2	January 17 & 19	Systems Biology (Networks & Emergence)
Week 3	January 24 & 26	Systems Biology (Components: DNA to Phenotype)
Week 4	Jan 31 & Feb 2	Systems Biology (Genomics / Technology)
Week 5	February 7 & 9	Epigenetics (History / Molecular Processes)
Week 6	February 14 & 16	Epigenetics (Molecular Processes & Integration)
Week 7	February 21 & 23	Epigenetics (Genomics and Technology)
Week 8	Feb 28 & March 2	Cell & Developmental Biology
Week 9	March 7 & 9	Epigenetics of Cell & Developmental Biology (& Midterm Exam)
Week 10	**March 13 – 17**	**Spring Break**
Week 11	March 21 & 23	Environmental Impact on Biology
Week 12	March 28 & 30	Environmental Epigenetics
Week 13	April 4 & 6	Disease Etiology
Week 14	April 11 & 13	Epigenetics & Disease Etiology
Week 15	April 18 & 20	Evolutionary Biology & Genetics
Week 16	April 25 & 27	Epigenetics & Evolutionary Biology
Week 17	May 2 & 4	Grant Review/ Study Section Meeting (& Final Exam)

Graduate Students

Grant Proposal

Outline:
- Title
- Abstract
- Specific Aims
- Background
- Preliminary Results
- Experimental Design and Methods
- References

(5-10 pp. single spaced typed limit)

Key Points:
- Specific aims should be focused and concise and clarify hypothesis
- Be as concise and direct as possible
- Work significance of proposal into grant when appropriate
- Use only critical preliminary results

Additional Information:
- Propose short-range studies to address long-range goals
- Write grant for 3 to 4 year period to complete studies
- Feasibility of success is critical, ask right type of question
- Experimental design needs to address hypothesis

Score/Rating:

Factors involved: Type question addressed, organization of thoughts, preliminary results, feasibility, reasonable completion expectations, focus of aims and proposed studies.

Score		
1.0 - 1.5	**Outstanding**	Funded
1.5 - 2.0	**Excellent**	Probably Funded
2.0 - 2.5	**Good**	Accepted, but not Fund
2.5 - 3.0	**Satisfactory**	
3.0 - 3.5	**Adequate**	
3.5 - 4.0	**Fair**	
4.0 - 5.0	**Acceptable**	

Review:

NIH Study Section style review with all students/fellows participating in the review. Primary and secondary reviewers will be selected and all grants will be critiqued.

Spring 2023 – Epigenetics and Systems Biology
Lecture Outline (Systems Biology)
Michael K. Skinner – Biol 476/576
Weeks 11 and 12 (March 2023)

Environmental Epigenetics

- Environmental Impacts on Biology
- Environment and Phenotype Variation
- Environmental Factors
- Environmental Epigenetics and Twin Studies
- Early life Exposures and Developmental Effects
- Nutrition and Epigenetics
- Environmental Toxicants and Epigenetics
- Environmental Induced Epigenetic Transgenerational Inheritance

Required Reading

Nilsson EE, Ben Maamar M, Skinner MK. Role of epigenetic transgenerational inheritance in generational toxicology. Environ Epigenet. 2022 Feb 16;8(1):dvac001. (PMID: 35186326)

Books (Reserve in Library)

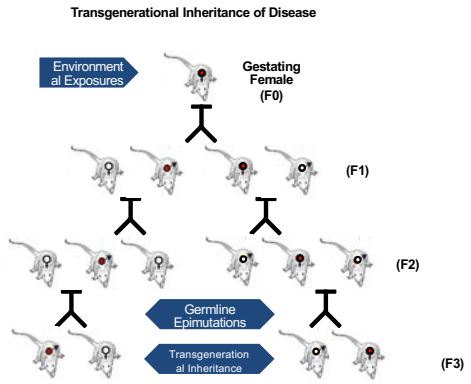
Scott F. Gilbert and David Epel (2009) Ecological Developmental Biology. Sinauer Associates Inc. Sunderland, Massachusetts.

E-Book: Craig and Wong (2011) Epigenetics: A Reference Manual. Caister Academic Press. ISBN-13: 978-1904455882.

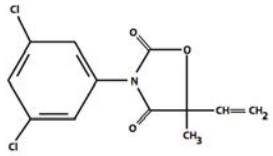
Environmental Epigenetics (Transgenerational Inheritance)

Epigenetic Transgenerational Inheritance Definition

- Germ line transmission of epigenetic marks in the absence of any continued direct environmental exposure to promote the generational inheritance of disease and phenotypic variation
- Distinct from direct exposure somatic or germ line epigenetic alterations not permanently programmed in the germ line

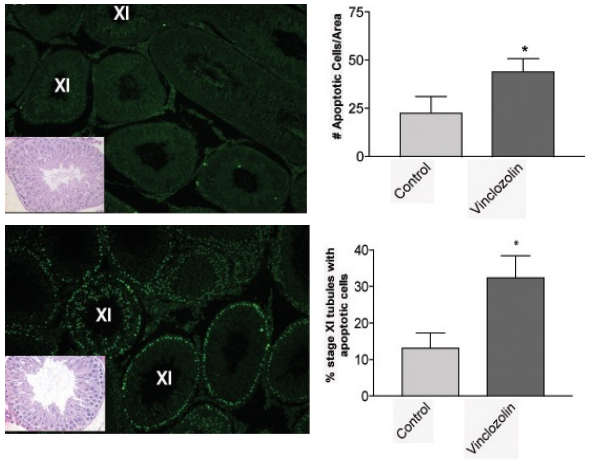


Model Endocrine Disruptor: Vinclozolin

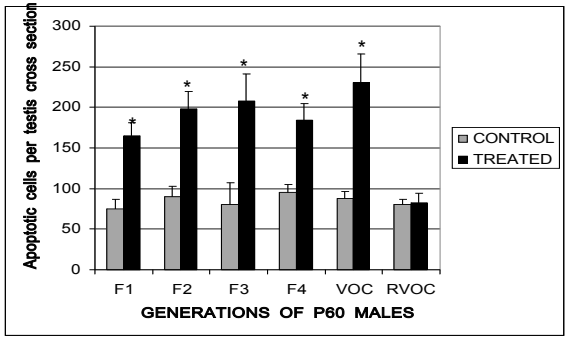


- Vinclozolin is a systemic fungicide (e.g. Wine Industry)
- Vinclozolin and its metabolites are anti-androgenic
- Late embryonic/early postnatal exposure causes abnormal reproductive tract development and gonadal function

Vinclozolin Effects on Spermatogenic Cell Apoptosis



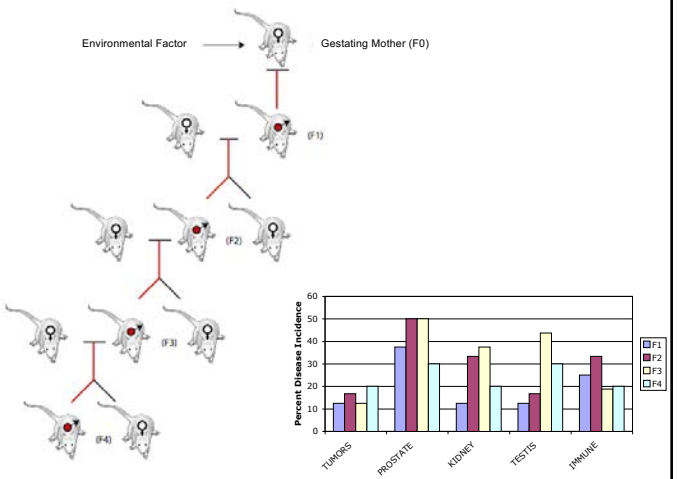
Germ Cell Apoptosis



* P<0.05

Vinclozolin

Transgenerational Inheritance of Disease

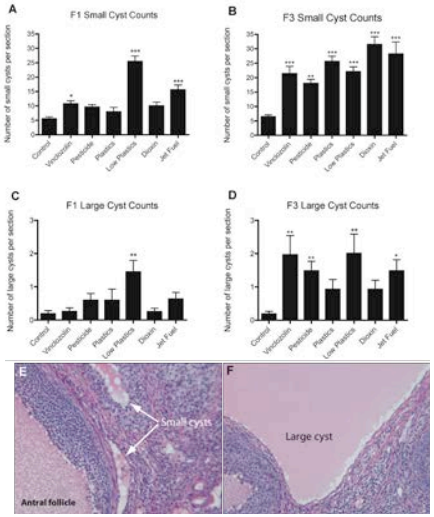


Compound Specificity

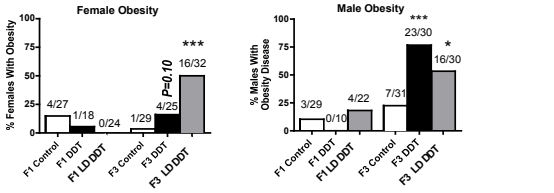
Environmental Compound Specificity

(Exposure Groups)	(Direct) F1	F3 (Transgenerational)
A. Vinclozolin [agricultural fungicide]	Yes	Yes
B. Flutamide [anti-androgenic pharmaceutical]	Yes	No
C. TCDD/Dioxin (industrial pollutant)	Yes	Yes
D. Plastics Compounds [Bisphenol-A BPA, Phthalate-DEHP & DBP]	Yes	Yes
E. Jet Fuel [JP8] (Hydrocarbon Mixture)	Yes	Yes
F. Pesticide & Insect Repellent [Permethrin & DEET]	No	Yes
G. DDT(pesticide)	Yes	Yes
H. Methoxychlor (pesticide, replace DDT)	Yes	Yes
I. Mercury (Industrial pollutant)	Yes	Yes
J. Atrazine (agricultural herbicide)	No	Yes
K. Glyphosate (pesticide herbicide)	No	Yes

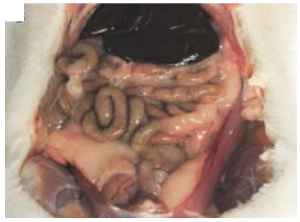
Polycystic Ovarian Disease



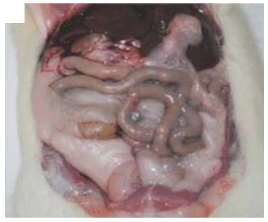
DDT Induced Transgenerational Obesity



Non-obese



Obese



TRANSGENERATIONAL DISEASE ETIOLOGY

MALE	FEMALE

- Spermatogenic Defect (>90%)
- Male infertility (complete ~10%, severe 20%)
- Prostate disease (~50%)
- Kidney disease (~30-40%)
- Increase in mammary tumor formation (~10-20%)
- Behavior (Mate Preference, Anxiety, & Stress) (>90%)
- Obesity (~10-50%)
- Pre-eclampsia-like during late pregnancy (~10%)
- Premature Ovarian Failure POF (>90%)
- Ovarian Polycystic Ovarian Disease (>90%)
- Female Premature Pubertal Onset (>90%)

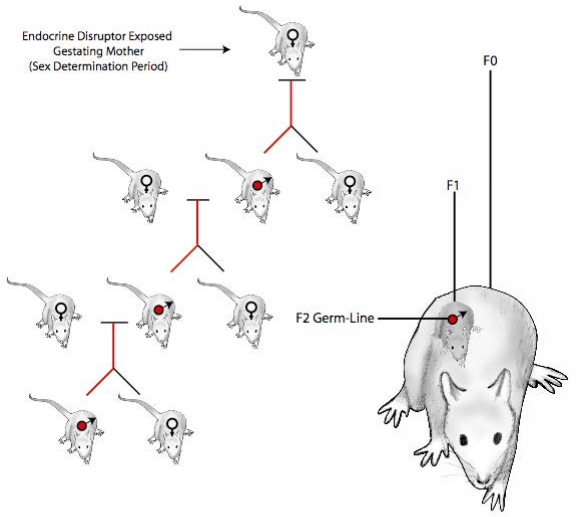
ENVIRONMENTALLY INDUCED EPIGENETIC TRANSGENERATIONAL INHERITANCE

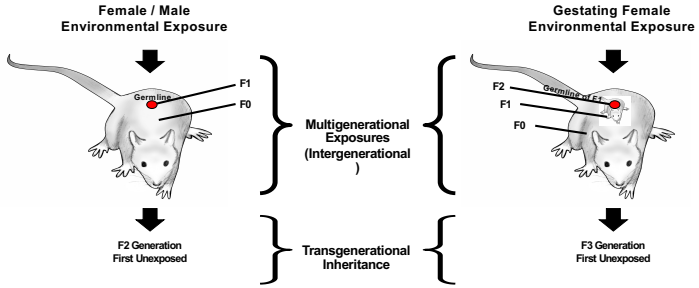
Environmental Toxicants

Vinclozolin (Agricultural Fungicide)	Permethrin & DEET (Insect Repellants)
Methoxychlor (Agricultural Pesticide)	DDT (Pesticide)
Dioxin/TCDD (Industrial Contaminant)	Tributyltin (Industrial Toxicant & Biocide)
Plastic Compounds (BPA & Phthalates)	Hydrocarbons (Jet Fuel)
Atrazine (Herbicide)	Glyphosate (Pesticide / Herbicide)

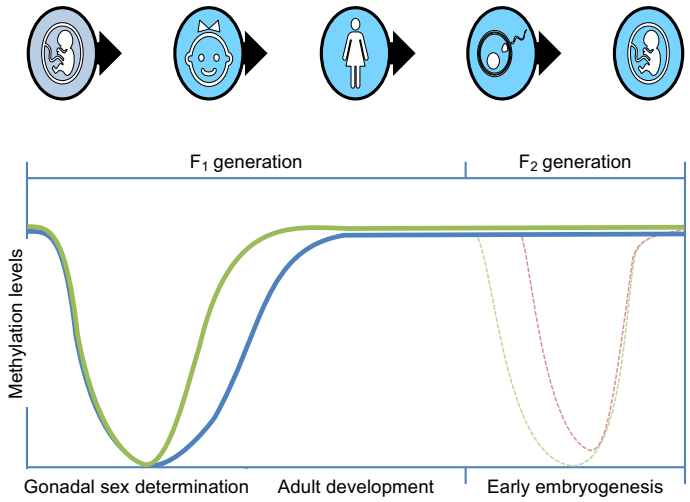
Other Types Exposures

Nutrition (High Fat or Caloric Restriction)	Smoking & Alcohol
Temperature & Drought (Plant Health & Flowering)	Stress Trauma (Behavioral)



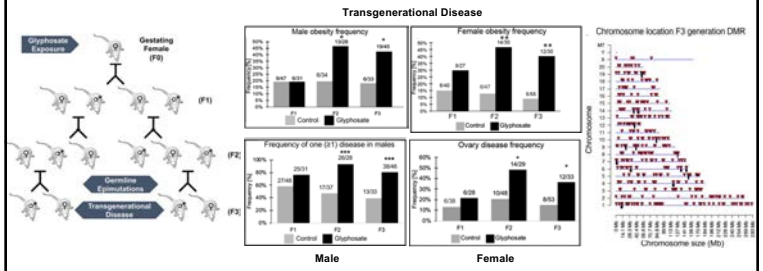


Environmentally induced transgenerational epigenetic inheritance: Schematic of environmental exposure and affected generations. Skinner (2008) Repro Tox.

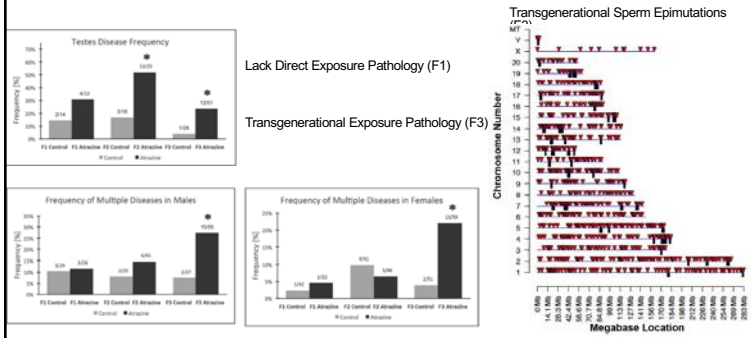


Assessment of Glyphosate Induced Epigenetic Transgenerational Inheritance of Pathologies and Sperm Epimutations: Generational Toxicology

Kubsad D, Nilsson EE, King SE, Sadler-Riggleman I, Beck D, Skinner MK.
Sci Rep. 2019, 9(1):6372.



PLOS ONE **McBirney M, King SE, Pappalardo M, Houser E, Unkefer M, Nilsson E, Sadler-Riggleman I, Beck D, Winchester P, Skinner MK. (2017) Atrazine induced epigenetic transgenerational inheritance of disease, lean phenotype and sperm epimutation pathology biomarkers. PLoS One. 12(9):e0184306.**

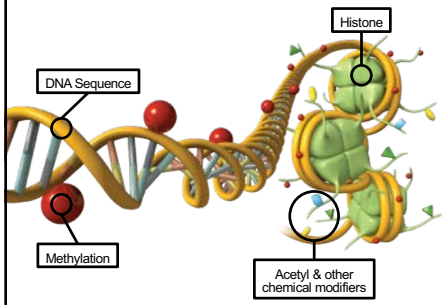


Lack Direct Exposure Pathology (F1)

Transgenerational Exposure Pathology (F3)

Need to Examine Transgenerational Pathology for Risk Assessment!!

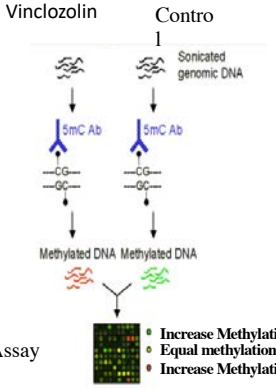
GERMLINE TRANSMITTED EPIGENETIC TRANSGENERATIONAL INHERITANCE



EPIGENETIC MECHANISMS AND MARKS

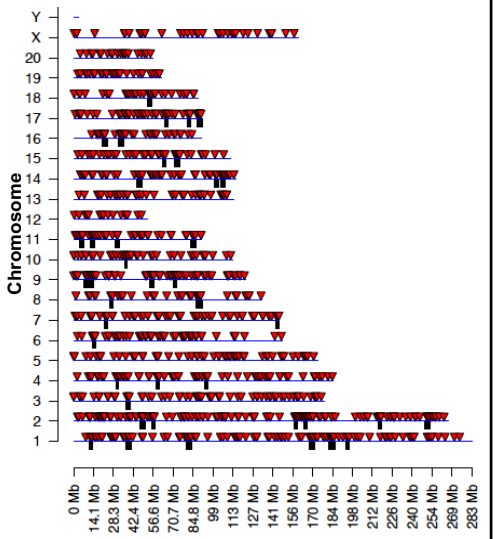
- **DNA Methylation**
- **Histone Modifications**
- **Non-coding RNA**

Comparative Methylation, MeDIP Chip or MeDIP Seq F3 Generation Sperm DNA pools



MeDIP-Chip Assay
or
MeDIP-Seq Assay

Next Generation Sequencing (linker bar codes)

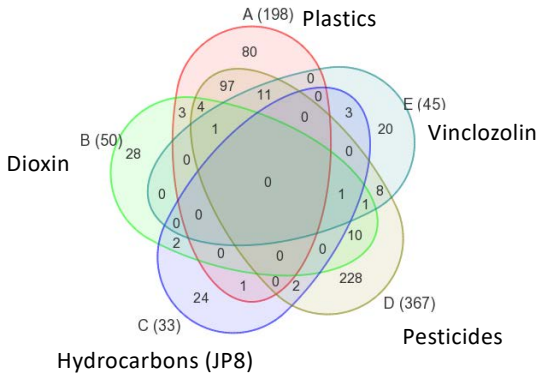


F3 Generation Sperm Genome Wide Epimutations (MeDIP-Seq)

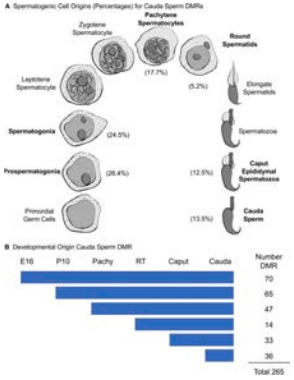
1075 p<10-4 DMR Sites

Transgenerational Epimutations & Clusters

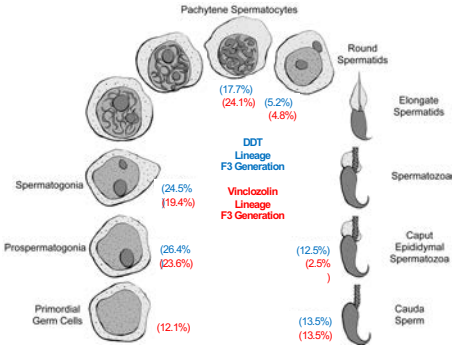
Exposure Specific Transgenerational (F3) Sperm Epigenome Alterations



Developmental origins of transgenerational sperm DNA methylation epimutations following ancestral DDT exposure.
Dev Biol. 2019 Jan 15;445(2):280-293.
Ben Maamar M, Nilsson E, Sadler-Riggleman I, Beck D, McCarrey JR, Skinner MK.



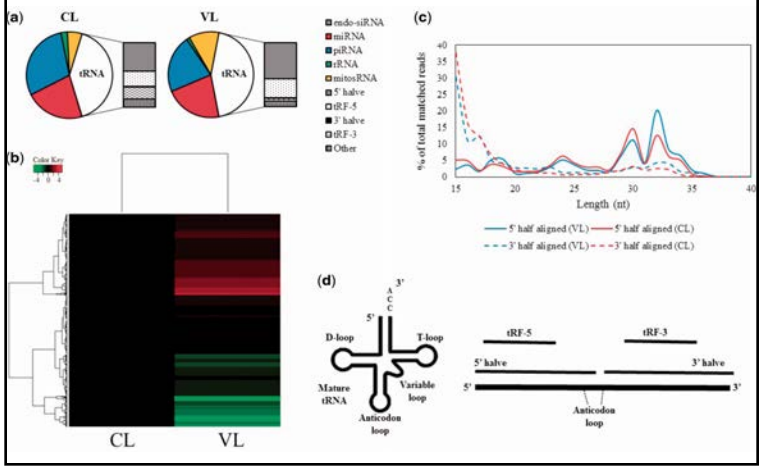
Developmental Origins of Sperm Epimutations (DMRs)



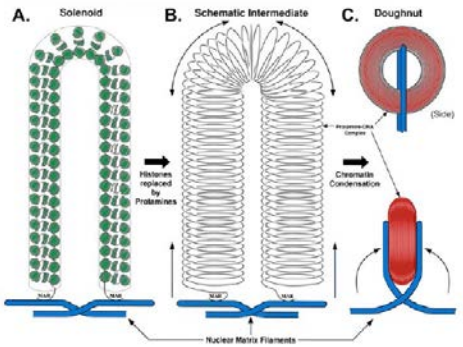
Collaborator- John McCarrey, UTSA

Ben Maamar, et al. (2019) Dev Biol 15;445(2):280-293
Skinner, et al (2019) Epigenetics14(7):721-739

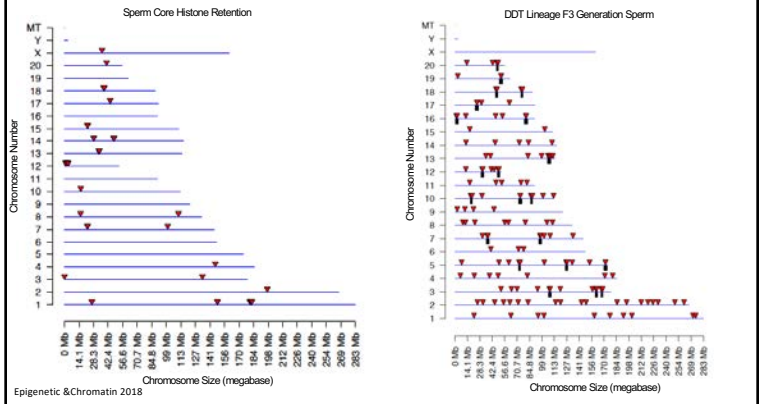
Ancestral vinclozolin exposure alters the epigenetic transgenerational inheritance of sperm small noncoding RNAs.
Environ Epigenet. 2016;2(1).
Schuster A, Skinner MK, Yan W.



Chromatin Structure – Composition and Function during Spermiogenesis
Khochbin, S. and Ward, W.S

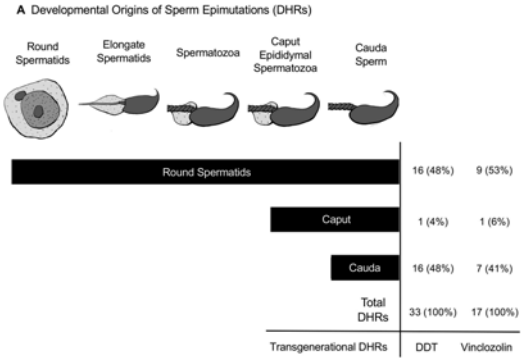


Environmentally Induced New Transgenerational Histone Retention

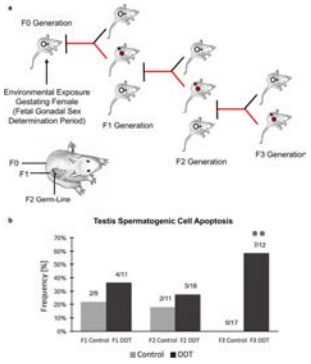


Epigenetic &Chromatin 2018

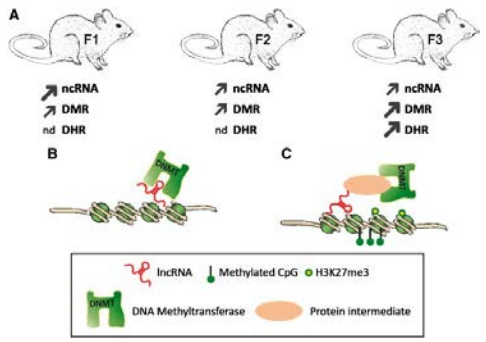
Developmental origins of transgenerational sperm histone retention following ancestral exposures.
Ben Maamar M, Beck D, Nilsson E, McCarrey JR, Skinner MK.
Dev Biol. 2020 Sep 1;465(1):31-45.



Alterations in sperm DNA methylation, non-coding RNA and histone retention associate with DDT-induced epigenetic transgenerational inheritance of disease.
Epigenetics Chromatin. 2018 Feb 27;11(1):8.
Skinner MK, Ben Maamar M, Sadler-Riggleman I, Beck D, Nilsson E, McBirney M, Klukovich R, Xie Y, Tang C, Yan W.

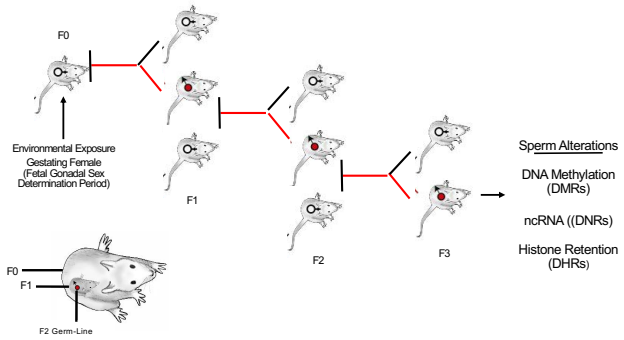


Alterations in sperm DNA methylation, non-coding RNA expression, and histone retention mediate vinclozolin-induced epigenetic transgenerational inheritance of disease.
Environ Epigenet. 2018 Apr 26;4(2):dvy010.
Ben Maamar M, Sadler-Riggleman I, Beck D, McBirney M, Nilsson E, Klukovich R, Xie Y, Tang C, Yan W, Skinner MK.

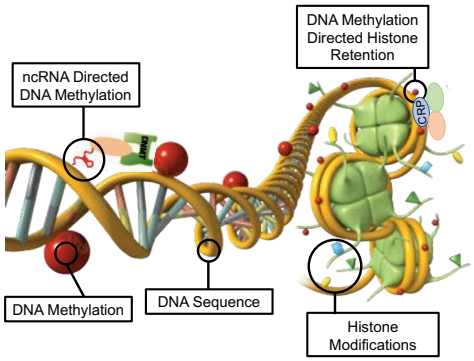


The regulatory mechanisms of DNA methylation by lncRNAs. **(A)** Summary of the different epimutations in the F1, F2, and F3 generation exposed rat sperm with larger/thicker arrows reflecting larger numbers of epimutations than thinner arrows and nd (negligible detected). **(B)** lncRNA/DNMT interaction prevents locus-specific DNA methylation locally *in cis*.**(C)** lncRNA interacts with DNMT1 (methyltransferase 1) indirectly through a protein intermediate.

Epigenetic Transgenerational Inheritance of Sperm Epimutations



Integration of Sperm ncRNA Directed DNA Methylation and DNA Methylation Directed Histone Retention in Transgenerational Inheritance
Beck D, Ben Maamar M, and Skinner MK
Epigenetics and Chromatin (2021) 14:6



Transgenerational (F3) Sperm Epigenome Mapping
(Germline transmitted epimutations)

(Epigenetic Biomarkers for Ancestral Exposures)

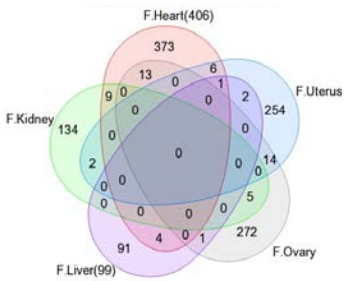
Transgenerational Sperm Epigenome Alterations
(>1000 differential DNA methylation sites)

Genomic Features-CpG Deserts (<10%) & Motifs (EDM1/2)
(Susceptibility epigenetic transgenerational mark)

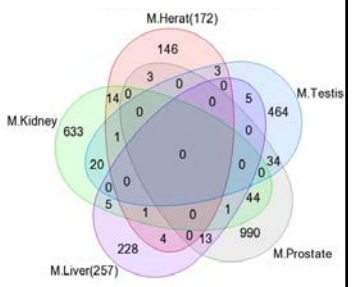
Genome Activity Alterations?
(transcriptome)

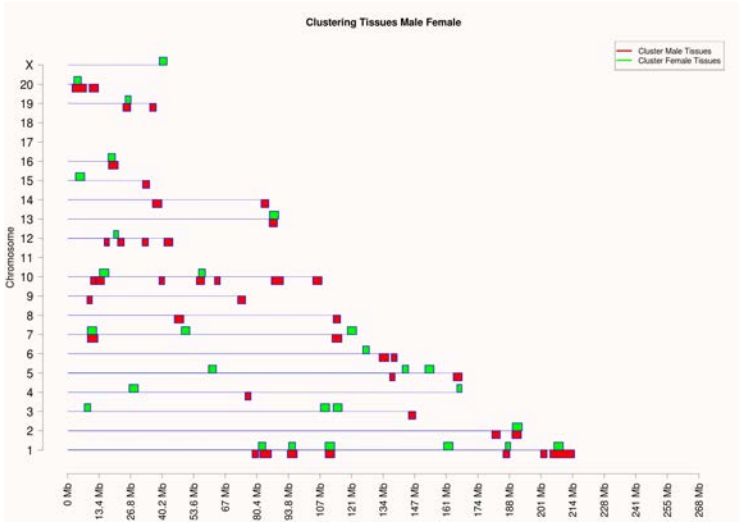
Tissue Specific Transgenerational Transcriptomes (F3)

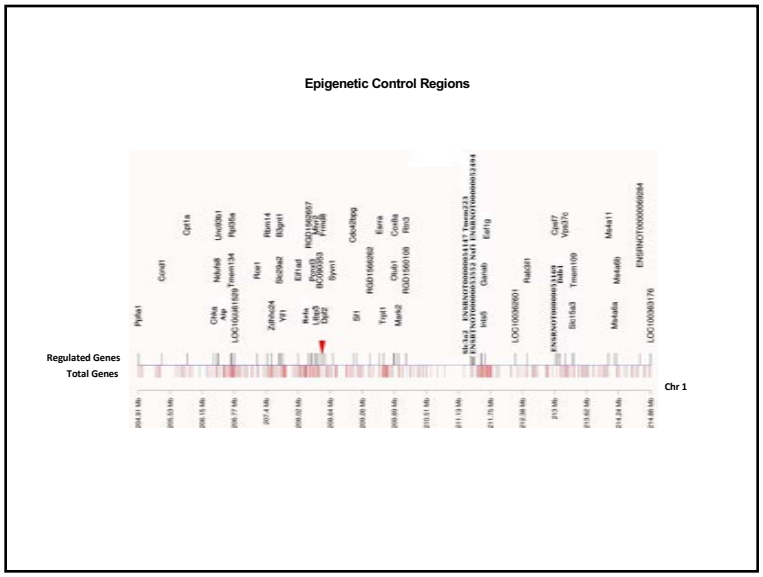
Female



Male



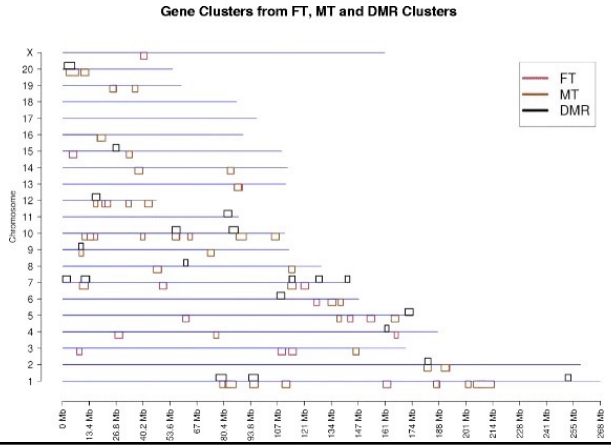


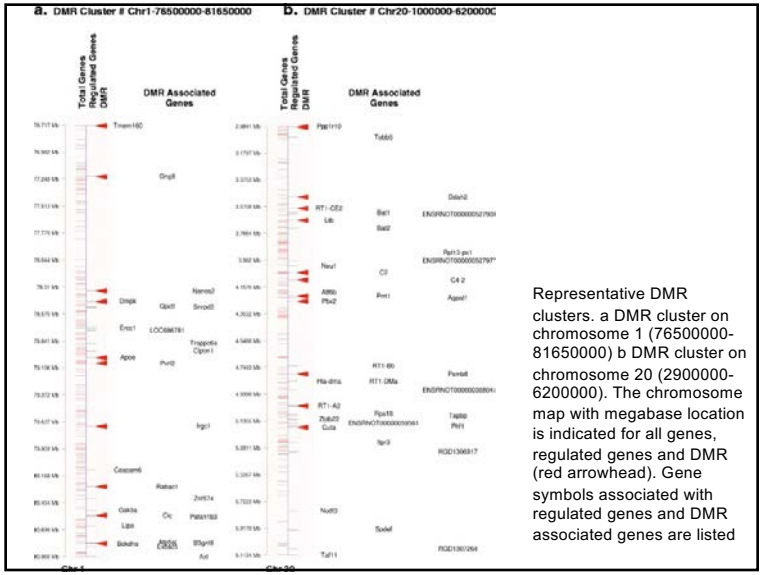


Genomic Clustering of differential DNA methylated regions (epimutations) associated with the epigenetic transgenerational inheritance of disease and phenotypic variation.

BMC Genomics. 2016 Jun 1;17:418.

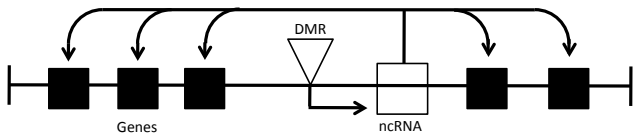
Haque MM, Nilsson EE, Holder LB, Skinner MK.



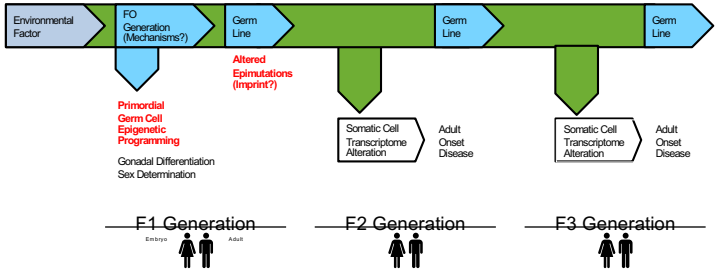


Representative DMR clusters. a DMR cluster on chromosome 1 (76500000-81650000) b DMR cluster on chromosome 20 (2900000-6200000). The chromosome map with megabase location is indicated for all genes, regulated genes and DMR (red arrowhead). Gene symbols associated with regulated genes and DMR associated genes are listed

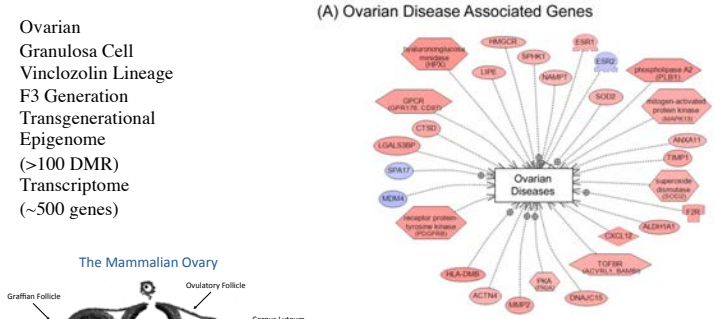
Epigenetic Control Region (2-5 megabase)

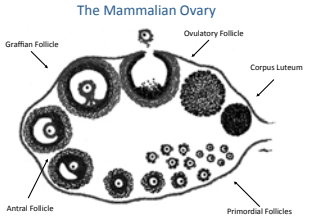


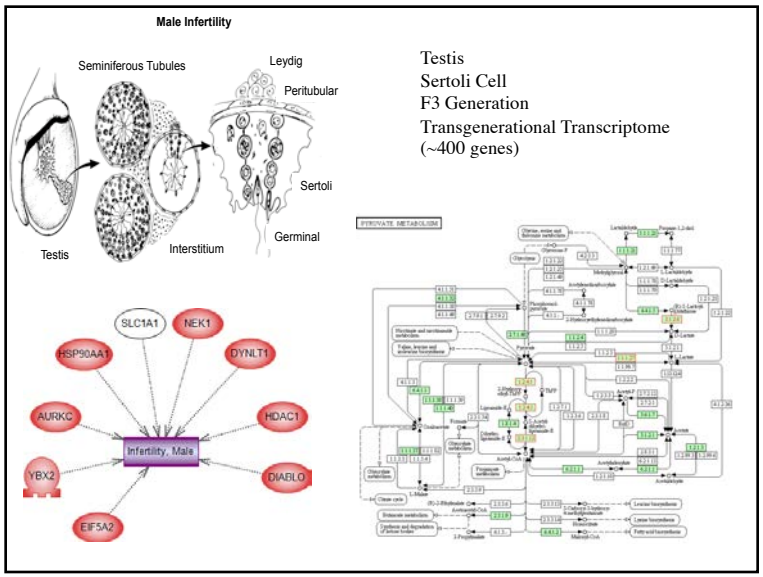
ROLE OF GERM LINE IN EPIGENETIC TRANSGENERATIONAL INHERITANCE



Ovarian
Granulosa Cell
Vinclozolin Lineage
F3 Generation
Transgenerational
Epigenome
(>100 DMR)
Transcriptome
(~500 genes)

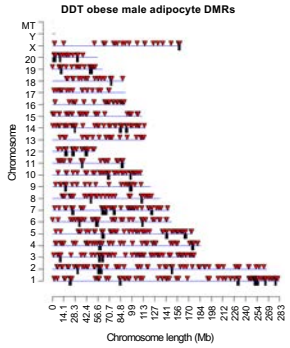


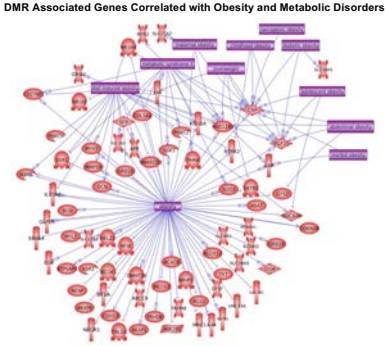




Testis
Sertoli Cell
F3 Generation
Transgenerational Transcriptome
(~400 genes)

Adipocyte Epigenetic Alterations and Potential Therapeutic Targets in Transgenerationally Inherited Lean and Obese Phenotypes Following Ancestral Exposures
King SE, Nilsson E, Beck D, Skinner MK
Adipocyte 2019 8(1) 362-378





EPIGENETIC TRANSGENERATIONAL INHERITANCE

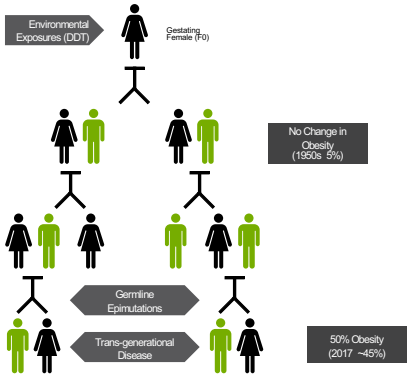
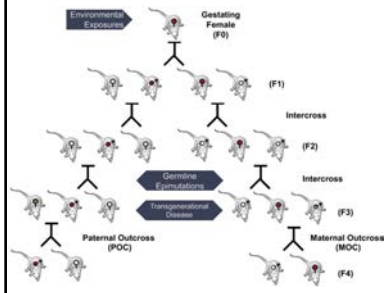


TABLE 1. Environmental exposures that induce transgenerational reproductive disease phenotypes.

Toxicants	Reproductive disease	References
Vinclozolin	Decreased sperm count, testis apoptosis, testis abnormalities,[a] prostate abnormalities, oocyte loss, ovarian cysts, altered mate selection. Epigenetic changes observed.	[51, 55–57, 63, 69, 78]
Methoxychlor	Ovarian cysts. Epigenetic changes observed.	[55, 75, 80]
TCDD/dioxin	Puberty onset, oocyte loss, ovarian cysts, fertility defect.[b] Epigenetic changes observed.	[72, 77, 78]
Plastics mixture (bisphenol-A, phthalate-DEHP, and DBP)	Testis abnormalities, puberty onset, oocyte loss, ovarian cysts. Epigenetic changes observed.	[67, 68]
Jet fuel (JP8)	Testis apoptosis, oocyte loss. Epigenetic changes observed.	[65, 74]
Permethrin and DEET	Testis abnormalities, puberty onset, oocyte loss, ovarian cysts. Epigenetic changes observed.	[69]
DDT	Decreased sperm count, testis apoptosis, ovarian cysts. Epigenetic changes observed.	[63, 64]
Bisphenol A	Decreased sperm count, fertility defect	[79, 89, 90]
Phthalates	Decreased sperm count, testis abnormalities, puberty onset, fertility defect	[66]
Tributyltin		[91]
Benzo[a]pyrene	Testis abnormalities	[70]
Other types exposures		
Folate (nutrition)		[92]
High-fat diet (nutrition)		[93, 94]
Caloric Restriction (nutrition)		[95–98]
Temperature and drought (plant flowering and health)	Abnormal flowering, fertility defect. Epigenetic changes observed.	[99–102]
Stress (behavioral)		[103, 104]
Smoking (health)		[105, 106]
Alcohol (health)		[107]

[a] Includes seminiferous tubule atrophy, tubule vacuoles, and germ cell agenesis.
[b] Fertility defect indicates reduced numbers of offspring.

Epigenetic transgenerational inheritance of parent-of-origin allelic transmission of outcross pathology and sperm epimutations.
Ben Maamar M, King SE, Nilsson E, Beck D, Skinner MK.
Dev Biol. 2020 Feb 1;458(1):106-119.

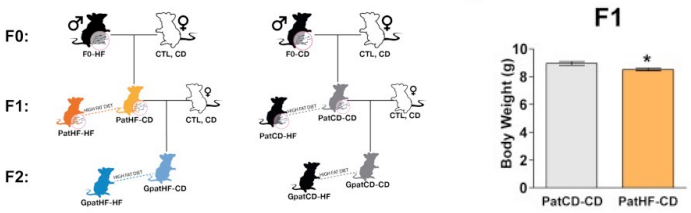


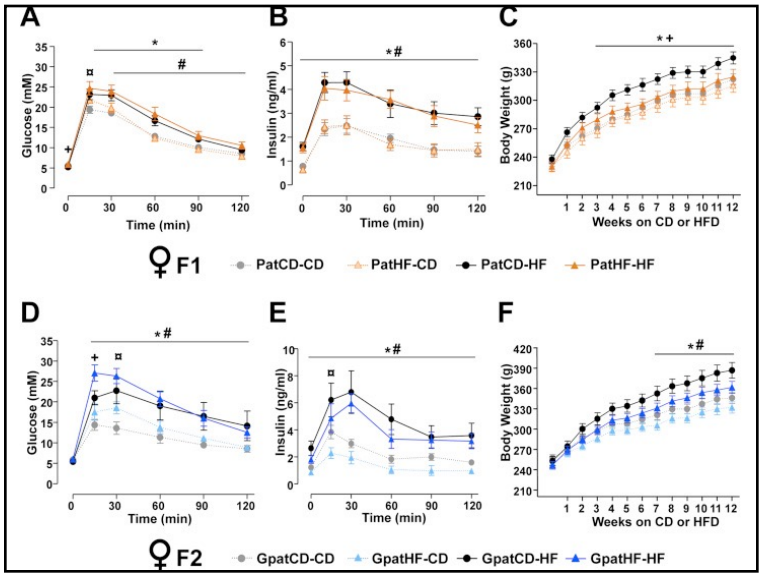
A Pathology parent-of origin-transmission

Diseases	F3 DDT	DDT F4 POC	DDT F4 MOC	DDT Allelic transmission	F3 Vinc	Vinc F4 POC	Vinc F4 MOC	Vinc Allelic transmission
Testis	+	-	-	Both alleles	+	-	-	Both allele
Ovary	+	-	-	Both alleles	-	-	-	No allele
Prostate	+	-	-	Both alleles	+	-	+	Maternal allele
Male Kidney	-	-	-	No allele	+	+	+	Either allele
Female Kidney	+	+	-	Paternal allele	-	+	-	Paternal allele
Male obesity	+	+	+	Either allele	-	+	+	Either allele
Female obesity	+	-	-	Both allele	+	+	-	Paternal allele
Male multiple diseases	+	-	+	Maternal allele	+	-	+	Maternal allele
Female multiple diseases	+	+	-	Paternal allele	+	+	-	Paternal allele
Female tumor	-	-	-	No allele	-	-	+	Maternal allele

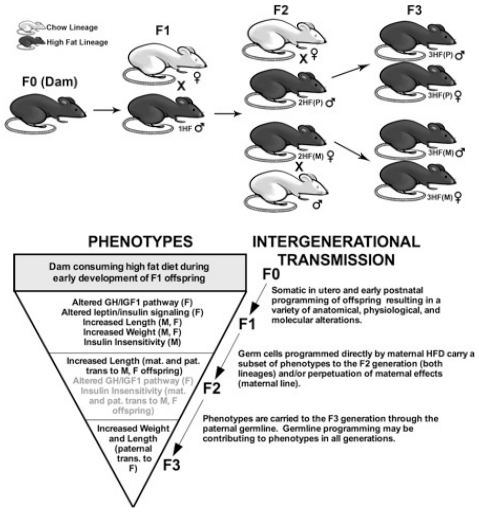
Diet Induced Epigenetic Transgenerational Inheritance

High-fat diet reprograms the epigenome of rat spermatozoa and transgenerationally affects metabolism of the offspring.
Mol Metab. 2015 Dec 25;5(3):184-97.
de Castro Barbosa T, Ingerslev LR, et al.

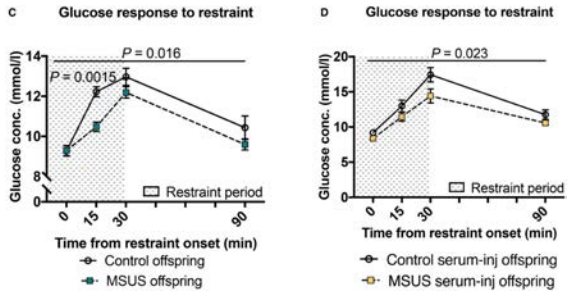




Dunn GA, Bale TL. (2011) Maternal high-fat diet effects on third-generation female body size via the paternal lineage. Endocrinology. 152(6):2228-36.

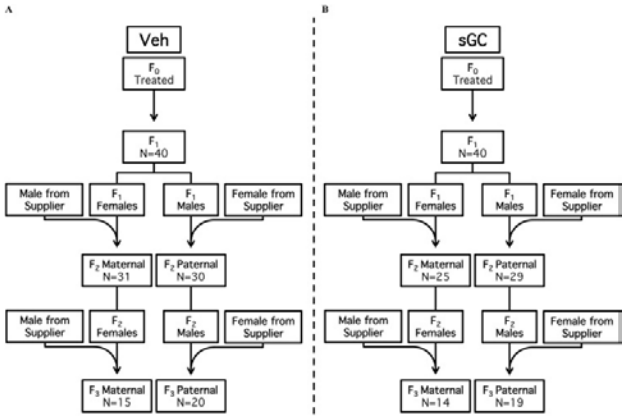


Involvement of circulating factors in the transmission of paternal experiences through the germline.
van Steenwyk G, Gapp K, Jawaid A, Germain PL, Manuella F, Tanwar DK, Zamboni N, Gaur N, Efimova A, Thumfart KM, Miska EA, Mansuy IM. EMBO J. 2020 Dec 1;39(23):e104579.



Toxicant & Chemical Exposures Induced Epigenetic Transgenerational Inheritance

Prenatal Glucocorticoid Exposure Modifies Endocrine Function and Behaviour for 3 Generations Following Maternal and Paternal Transmission.
Sci Rep. 2017 Sep 18;7(1):11814.
Moisiadis VG, Constantinof A, Kostaki A, Szyf M, Matthews SG.



Bruner-Tran KL, Osteen KG. (2011) Developmental exposure to TCDD reduces fertility and negatively affects pregnancy outcomes across multiple generations. Reprod Toxicol. 31(3):344-50.

Table 2
Impact of developmental TCDD exposure on reproductive outcome in MPV-free C57BL/6 mice over multiple generations.

Exposure	Pregnancy rate	Pregnancy outcome	
		Full-term	Preterm
Vehicle control[a]			
conF1	10/10 (100%)	10/10[b]	0/10
conF3	12/12 (100%)	12/12	0/12
TCDD *in utero*[c]			
F1	11/28 (39%)	7/11	4/11
F3	8/14 (57%)	6/8	2/8

[a] Pregnant mice were exposed to corn oil vehicle (control) on E15.5 and control offspring (conF1 mice) mated at 10–12 weeks of age. Offspring of conF1 mice (conF2 mice) were mated at a similar age, as were the conF3 mice.
[b] Only a subset of conF1–F3 offspring were used to obtain additional generations of unexposed mice.
[c] Pregnant mice were exposed to 10 μg/kg TCDD in corn oil vehicle on E15.5 and singly exposed offspring (F1 mice) were mated at 10–12 weeks of age. Offspring of singly exposed F1 mice (F2 mice) were mated at a similar age, as were the F3 mice.

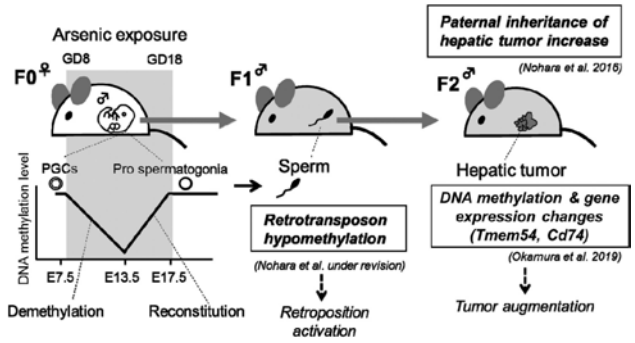
Prenatal Exposure to Environmentally-Relevant Contaminants Perturbs Male Reproductive Parameters Across Multiple Generations that are Partially Protected by Folic Acid Supplementation.
Lessard M, Herst PM, Charest PL, Navarro P, Joly-Beauparlant C, Droit A, Kimmins S, Trasler J, Benoit-Biancamano MO, MacFarlane AJ, Dalvai M, Bailey JL.
Sci Rep. 2019 Sep 25;9(1):13829.

Early-life exposure to POPs harms male reproduction across multiple generations. FA supplementation partly mitigated the impact of POPs. The two-cell embryo transcriptome is susceptible to paternal environment and could be the foundation for later pregnancy outcomes.

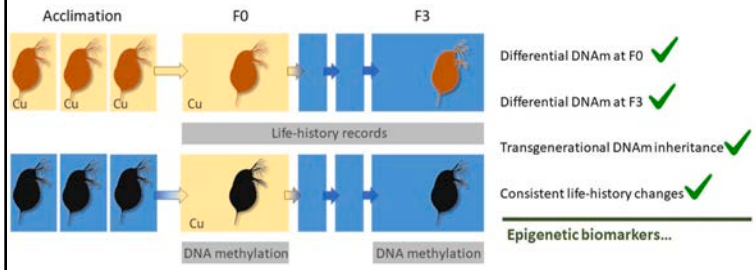
Association of Exposure to Diethylstilbestrol During Pregnancy With Multigenerational Neurodevelopmental Deficits.
Kioumourtzoglou MA, Coull BA, O'Reilly ÉJ, Ascherio A, Weisskopf MG.
JAMA Pediatr. 2018 Jul 1;172(7):670-677.

This study provides evidence that diethylstilbestrol exposure is associated with multigenerational neurodevelopmental deficits. The doses and potency level of environmental endocrine disruptors to which humans are exposed are lower than those of diethylstilbestrol, but the prevalence of such exposure and the possibility of cumulative action are potentially high and thus warrant consideration.

Gestational arsenic exposure and paternal intergenerational epigenetic inheritance.
Nohara K, Suzuki T, Okamura K.
Toxicol Appl Pharmacol. 2020 Dec 15;409:115319.

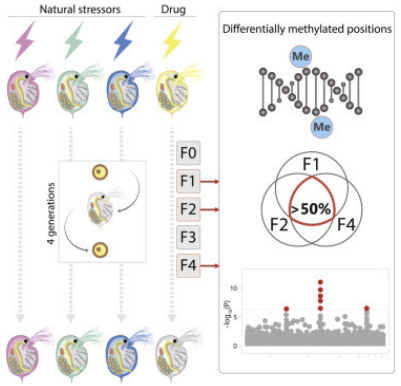


Multigenerational DNA methylation responses to copper exposure in Daphnia: Potential targets for epigenetic biomarkers?
Jeremias G, Veloso T, Gonçalves FJM, Van Nieuwerburgh F, Pereira JL, Asselman J.
Chemosphere. 2022 Dec;308(Pt 1):136231.



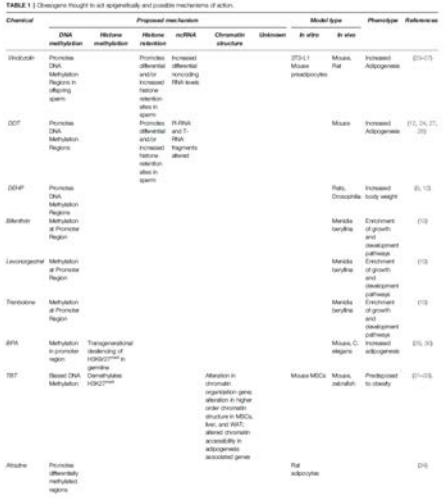
Highlights
- Direct and inherited effects of Cu in DNA methylation of *Daphnia* were explored.
- Methylation changes targeted genes that offset metal toxicity and oxidative stress.
- Distinct methylation effects noticed in daphnids differing in Cu exposure history.
- Exposure history promoted transgenerational inheritance in a specific manner.

Environmentally induced DNA methylation is inherited across generations in an aquatic keystone species.
Feiner N, Radersma R, Vasquez L, Ringnér M, Nystedt B, Raine A, Tobi EW, Heijmans BT, Uller T.
iScience. 2022 Apr 25;25(5):104303.



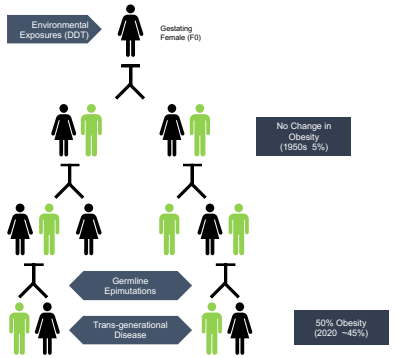
Highlights
- Naturally induced DNA-methylation persists until generation F4 in *Daphnia*
- Drug-induced de-methylation is reset after one generation
- Methylation is enriched in exons suggesting a gene regulatory function
- Epigenetic inheritance may influence eco-evolutionary dynamics

Epigenetic Transgenerational Inheritance of the Effects of Obesogen Exposure.
Mohajer N, Joloya EM, Seo J, Shioda T, Blumberg B.
Front Endocrinol (Lausanne). 2021 Dec 16;12:787580.



Epigenetic Transgenerational Inheritance of Obesity Susceptibility.
King SE, Skinner MK.
Trends Endocrinol Metab. 2020 Jul;31(7):478-494.

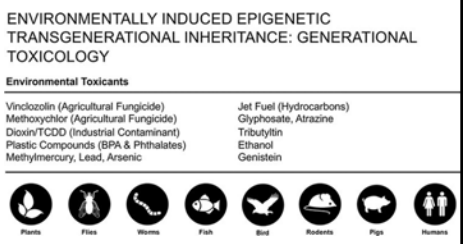
EPIGENETIC TRANSGENERATIONAL INHERITANCE OF OBESITY SUSCEPTIBILITY



Role of epigenetic transgenerational inheritance in generational toxicology.
Nilsson EE, Ben Maamar M, Skinner MK.
Environ Epigenet. 2022 Feb 16;8(1):dvac001.

Table 1: Environmental toxicant induction of epigenetic transgenerational inheritance: generational toxicology

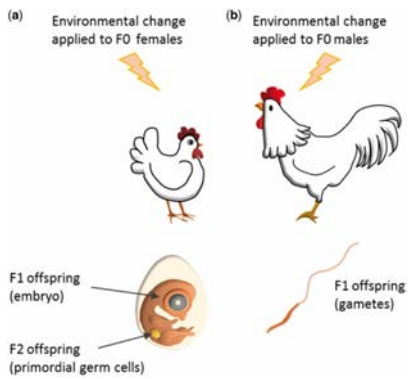
Toxicants	References
Vinclozolin	[47–51, 84, 85, 92, 95, 98, 99, 101, 103, 104]
TCDD/dioxin	[68]
Plastics compounds (BPA, phthalates DEHP and DBP)	[52–59]
Jet fuel (JP8) (hydrocarbon mixture)	[62]
Pesticides and insect repellent (permethrin and DEET)	[67]
DDT	[61, 87, 92, 96, 104]
Methoxychlor	[64]
Chlordecone	[102]
Methylmercury	[76]
Lead	[105]
Arsenic	[63, 70–74]
Atrazine	[64, 65]
Glyphosate	[86, 93]
Decabromodiphenyl ether (BDE-209)	[88]
Tributyltin	[60]
5-azacytidine	[77]
Ethanol	[75]
Benzo[a]pyrene	[69]
Genistein	[79]



Environmentally induced epigenetic transgenerational inheritance.

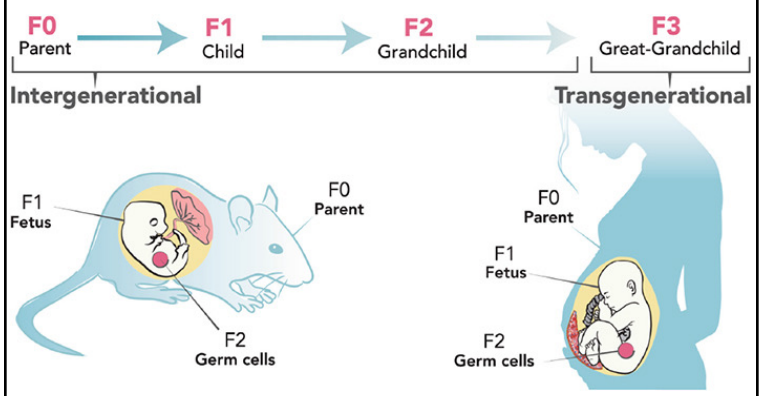
Stress Induced Epigenetic Transgenerational Inheritance

Transgenerational epigenetic inheritance in birds.
Environ Epigenet. 2018 Apr 26;4(2):dvy008.
Guerrero-Bosagna C, Morisson M, Liaubet L, Rodenburg TB, de Haas EN, Košťál Ľ, Pitel F.



The maternal environment directly impacts F1 and F2 offspring while the paternal environment only impacts F1 offspring. (**a**) A change in the maternal environment can affect egg components and thus may impact F1 individuals. However, as these F1 developing offspring bear the PGCs that will lead to differentiated gametes, the change in maternal environment may also impact F2 individuals. Thus only the effects observed on the F3 individuals will be considered as transgenerational effects. (**b**) A change in the paternal environment only affects its own gametes that will lead to the F1 generation. The effects observed on the F2 individuals will be considered as transgenerational effects

Prenatal maternal stress and offspring aggressive behavior: Intergenerational and transgenerational inheritance.
Mbiydzenyuy NE, Hemmings SMJ, Qulu L.
Front Behav Neurosci. 2022 Sep 23;16:977416.

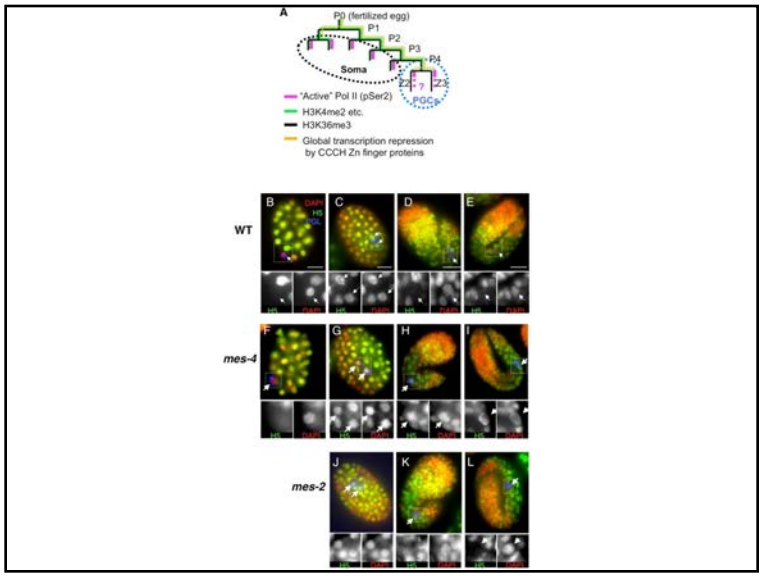


Other Inducers Epigenetic Transgenerational Inheritance

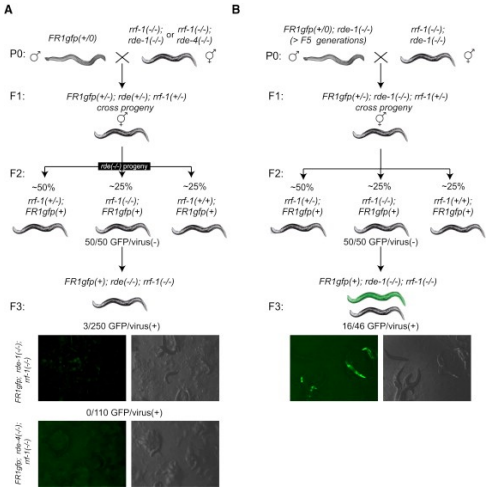
Trans-generational epigenetic regulation of C. elegans primordial germ cells.

Furuhashi H, Takasaki T, Rechtsteiner A, Li T, Kimura H, Checchi PM, Strome S, Kelly WG.

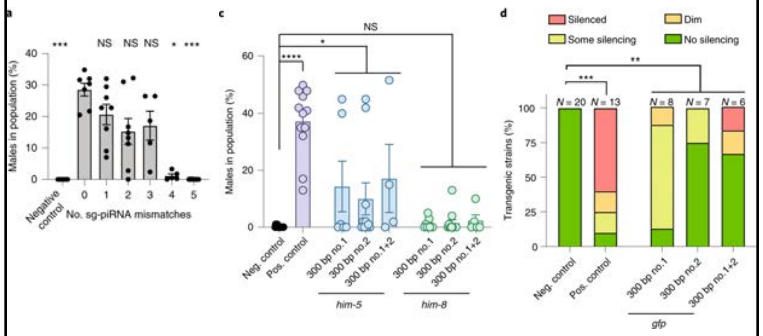
Epigenetics Chromatin. 2010 Aug 12;3(1):15.



Rechavi O, Minevich G, Hobert O. (2011) Transgenerational inheritance of an acquired small RNA-based antiviral response in C. elegans. Cell. 9;147(6):1248-56.

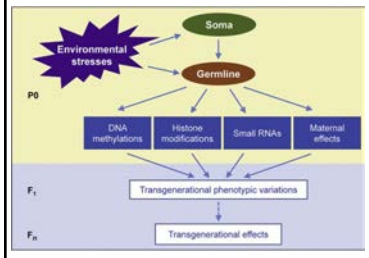


Reprogramming the piRNA pathway for multiplexed and transgenerational gene silencing in C. elegans.
Priyadarshini M, Ni JZ, Vargas-Velazquez AM, Gu SG, Frøkjær-Jensen C.
Nat Methods. 2022 Feb;19(2):187-194.

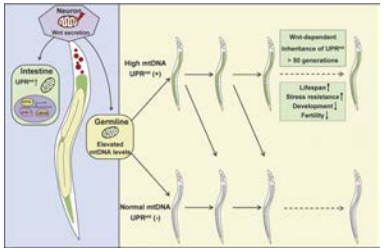


piRNAi tools for specific and scalable gene silencing.

Molecular insights into the transgenerational inheritance of stress memory.
Zhang Q, Tian Y.
J Genet Genomics. 2022 Feb;49(2):89-95.

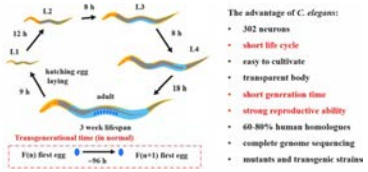


Environmental stresses can influence the phenotypes of offspring.

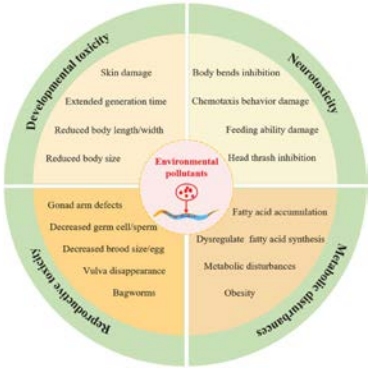


Transgenerational inheritance of increased mtDNA levels and the UPR^{mt}.

Review of the toxicity and potential molecular mechanisms of parental or successive exposure to environmental pollutants in the model organism Caenorhabditis elegans.
Li H, Zeng L, Wang C, Shi C, Li Y, Peng Y, Chen H, Zhang J, Cheng B, Chen C, Xiang M, Huang Y.
Environ Pollut. 2022 Oct 15;311:119927.

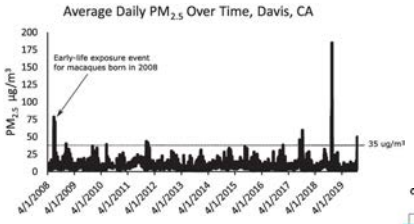


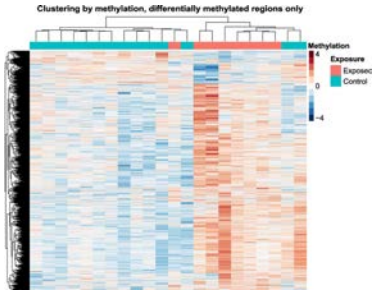
Advantages of using *C. elegans* as a model to evaluate toxicity from multi/transgenerational exposure to environmental pollutants.



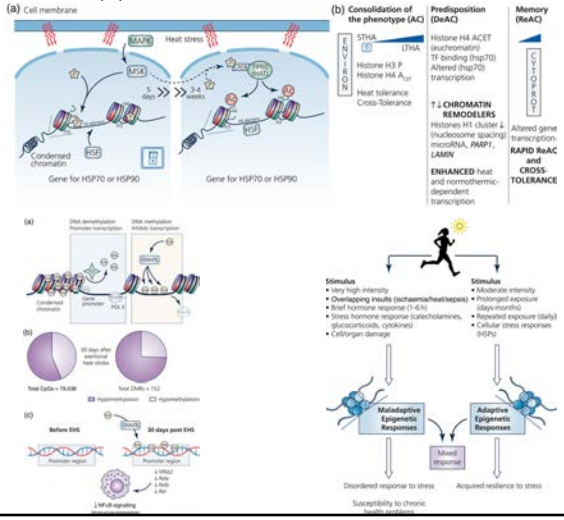
Multi/transgenerational toxicity in *C. elegans* induced by environmental pollutant exposure.

Long-term effects of wildfire smoke exposure during early life on the nasal epigenome in rhesus macaques.
Brown AP, Cai L, Laufer BI, Miller LA, LaSalle JM, Ji H.
Environ Int. 2022 Jan;158:106993.

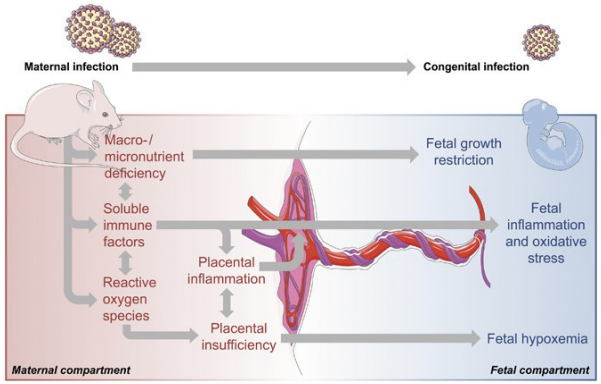




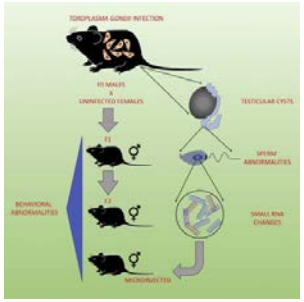
Epigenetic responses to heat: From adaptation to maladaptation.
Murray KO, Clanton TL, Horowitz M.
Exp Physiol. 2022 Oct;107(10):1144-1158.



Epigenetic and transgenerational mechanisms in infection-mediated neurodevelopmental disorders.
Weber-Stadlbauer U.
Transl Psychiatry. 2017 May 2;7(5):e1113.



Pathogenic Infection in Male Mice Changes Sperm Small RNA Profiles and Transgenerationally Alters Offspring Behavior
Shiraz Tyebji, Anthony J Hannan, Christopher J Tonkin
Cell Rep. 2020 Apr 28;31(4):107573.



Highlights
- F1 and F2 generation of *T. gondii*-infected males display behavioral abnormalities
- Offspring behavioral changes display sexual dimorphism
- *T. gondii* infection leads to changes in sperm small RNA levels
- Zygotic microinjection of isolated sperm small RNA recapitulates behavioral changes

Transgenerational inheritance of fetal alcohol exposure adverse effects on immune gene interferon-Ɣ
Omkaram Gangisetty, Ajay Palagani, Dipak K Sarkar
Clin Epigenetics. 2020 May 24;12(1):70.

Overall, these findings provide the evidence that fetal alcohol exposures produce an epigenetic mark on the Ifn-ɣ gene that passes through multiple generations via the male germ line. These data provide the first evidence that the male germ line transmits fetal alcohol exposure's adverse effects on the immune system.

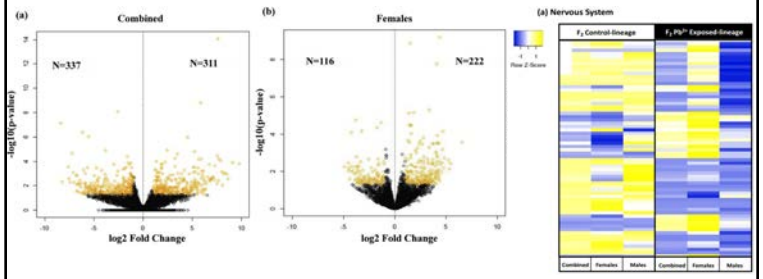
Sex-specific transgenerational effects of morphine exposure on reward and affective behaviors.
Brynildsen JK, Sanchez V, Yohn NL, Carpenter MD, Blendy JA.
Behav Brain Res. 2020 Oct 1;395:112842.

One generation later, affective behaviors were no longer altered in F2 males but F2 females from the F0 male morphine exposure buried more marbles in the MB test. In summary, early exposure to morphine in males and females causes lineage-specific inheritance of reward and affective behaviors.

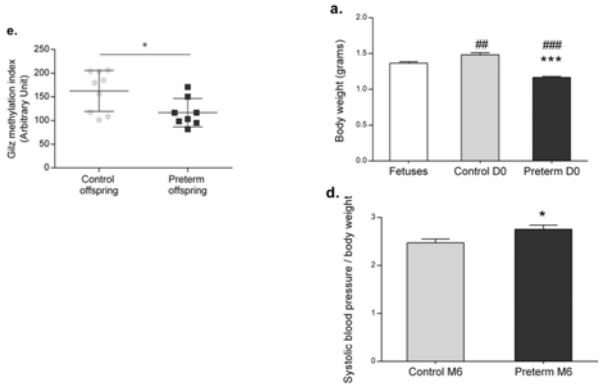
Epigenetic Responses to Temperature and Climate
Beth A McCaw, Tyler J Stevenson, Lesley T Lancaster
Integr Comp Biol. 2020 Dec 16;60(6):1469-1480.

Although the evidence points towards a conserved role of epigenetics in responding to temperature change, there appears to be an element of temperature- and species-specificity in the specific effects of temperature change on epigenetic modifications and resulting phenotypic responses. The review identifies areas of future research in epigenetic responses to environmental temperature change.

Developmental exposure to Pb 2+ induces transgenerational changes to zebrafish brain transcriptome
Danielle N Meyer, Emily J Crofts, Camille Akemann, et al.
Chemosphere. 2020 Apr;244:125527.

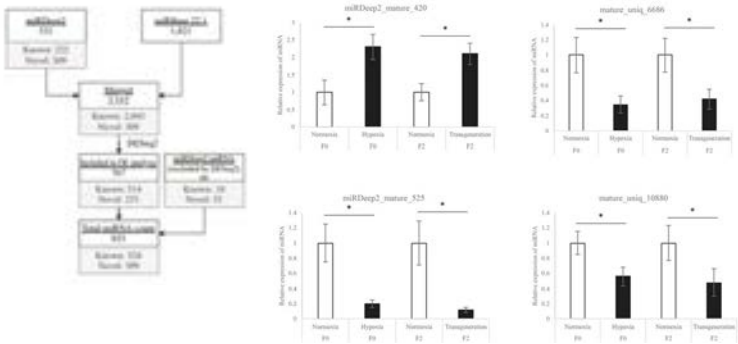


Preterm birth is associated with epigenetic programming of transgenerational hypertension in mice
Laurence Dumeige, Mélanie Nehlich, Say Viengchareun, Julie Perrot, Eric Pussard, Marc Lombès, Laetitia Martinerie
Exp Mol Med. 2020 Jan;52(1):152-165.

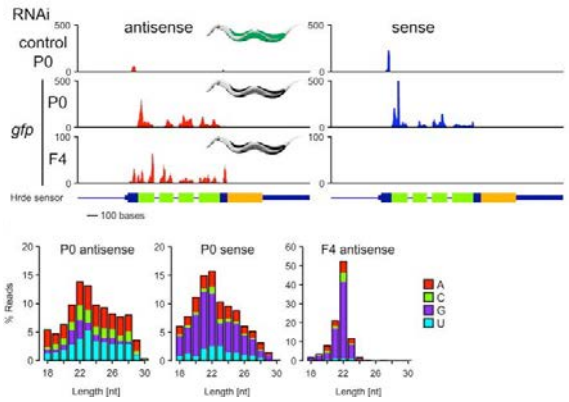


ncRNA Role Epigenetic Transgenerational Inheritance

Hypoxia-induced epigenetic transgenerational miRNAs dysregulation involved in reproductive impairment of ovary.
Lai KP, Tim Leung CC, Boncan DAT, Tam N, Lin X, Wang SY, Chan TF, Sun Wu RS, Chong Kong RY.
Chem Biol Interact. 2022 Nov 1;367:110176.

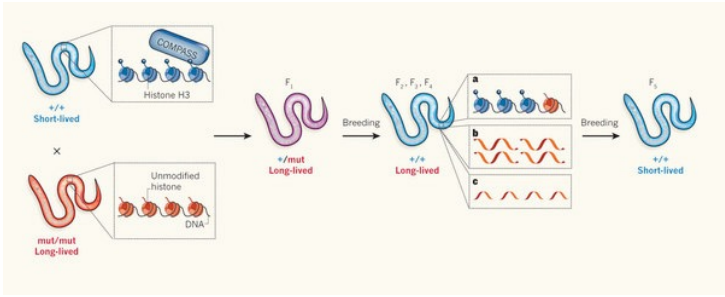


Ashe A, et al. (2012) piRNAs can trigger a multigenerational epigenetic memory in the germline of C. elegans. Cell. 6;150(1):88-99.

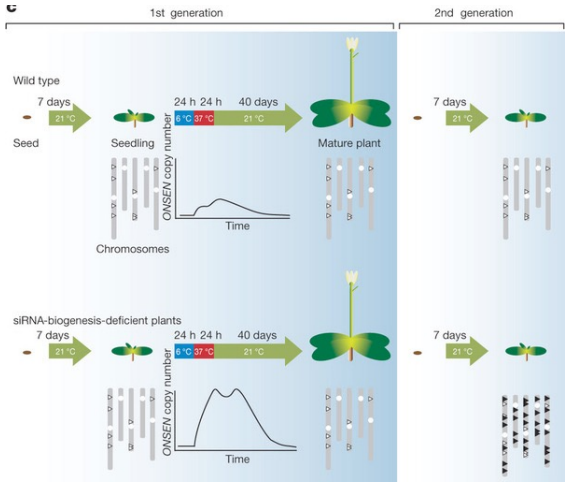


Mango SE. (2011) Ageing: generations of longevity. Nature. 16;479(7373):302-3.

The lifespan of some organisms can be extended by mutations that alter how DNA is packaged in their cells. A study reveals that this effect can last for generations, even in descendants that are genetically normal.



Ito H, et al. (2011) An siRNA pathway prevents transgenerational retrotransposition in plants subjected to stress. Nature. 7;472(7341):115-9.

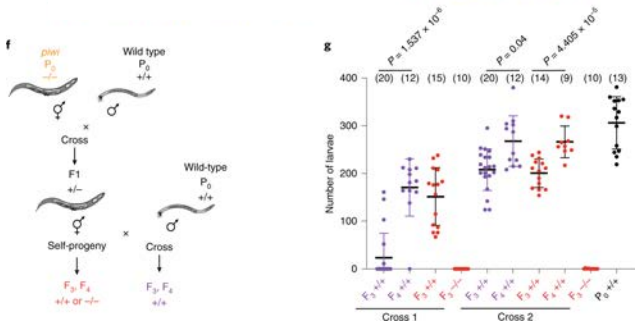


Small RNAs Reflect Grandparental Environments in Apomictic Dandelion.
Mol Biol Evol. 2017 Aug 1;34(8):2035-2040.
Morgado L, Preite V, Oplaat C, Anava S, Ferreira de Carvalho J, Rechavi O, Johannes F, Verhoeven KJF.

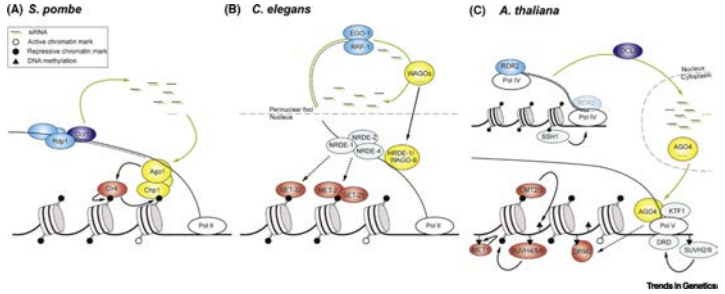
Abstract

Plants can show long-term effects of environmental stresses and in some cases a stress "memory" has been reported to persist across generations, potentially mediated by epigenetic mechanisms. However, few documented cases exist of transgenerational effects that persist for multiple generations and it remains unclear if or how epigenetic mechanisms are involved. Here, we show that the composition of small regulatory RNAs in apomictic dandelion lineages reveals a footprint of drought stress and salicylic acid treatment experienced two generations ago. Overall proportions of 21 and 24 nt RNA pools were shifted due to grandparental treatments. While individual genes did not show strong up- or downregulation of associated sRNAs, the subset of genes that showed the strongest shifts in sRNA abundance was significantly enriched for several GO terms including stress-specific functions. This suggests that a stress-induced signal was transmitted across multiple unexposed generations leading to persistent changes in epigenetic gene regulation.

Small-RNA-mediated transgenerational silencing of histone genes impairs fertility in piRNA mutants
Giorgia Barucci, Eric Cornes, Meetali Singh, Blaise Li, et al.
Nat Cell Biol. 2020 Feb;22(2):235-245.



Small RNAs in the Transgenerational Inheritance of Epigenetic Information
Lea Duempelmann, Merle Skribbe, Marc Bühler
Trends Genet. 2020 Mar;36(3):203-214.



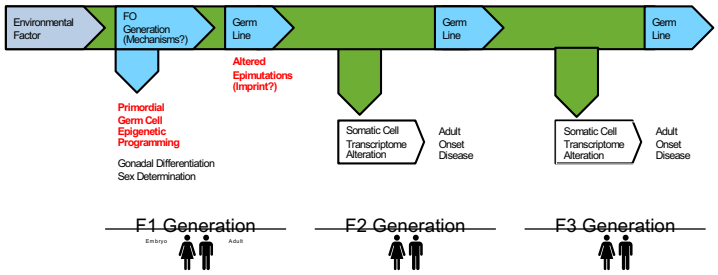
poly(UG)-tailed RNAs in genome protection and epigenetic inheritance
Aditi Shukla, Jenny Yan, Daniel J Pagano, et al.
Nature. 2020 Jun;582(7811):283-288.

Our results show that cycles of pUG RNA-templated siRNA synthesis and siRNA-directed pUG RNA biogenesis underlie double-stranded-RNA-directed transgenerational epigenetic inheritance in the C. elegans germline. We speculate that this pUG RNA-siRNA silencing loop enables parents to inoculate progeny against the expression of unwanted or parasitic genetic elements.

Small-RNA-mediated transgenerational silencing of histone genes impairs fertility in piRNA mutants
Giorgia Barucci, Eric Cornes, Meetali Singh, et al.
Nat Cell Biol. 2020 Feb;22(2):235-245.

PIWI-interacting RNAs (piRNAs) promote fertility in many animals. However, whether this is due to their conserved role in repressing repetitive elements (REs) remains unclear. Here, we show that the progressive loss of fertility in Caenorhabditis elegans lacking piRNAs is not caused by derepression of REs or other piRNA targets but, rather, is mediated by epigenetic silencing of all of the replicative histone genes. In the absence of piRNAs, downstream components of the piRNA pathway relocalize from germ granules and piRNA targets to histone mRNAs to synthesize antisense small RNAs (sRNAs) and induce transgenerational silencing. Removal of the downstream components of the piRNA pathway restores histone mRNA expression and fertility in piRNA mutants, and the inheritance of histone sRNAs in wild-type worms adversely affects their fertility for multiple generations. We conclude that sRNA-mediated silencing of histone genes impairs the fertility of piRNA mutants and may serve to maintain piRNAs across evolution.

ROLE OF GERM LINE IN EPIGENETIC TRANSGENERATIONAL INHERITANCE



Epigenetic Alterations Promote Genetic Instability

Genetic Mutation	Epigenetic Alteration	DNA Sequence Alteration
Point Mutation (SNP)	DNA Methylation (CpG)	Susceptibility C ➔ T Conversion
Copy Number Variation (CNV)	Hypomethylation (Repeats)	Susceptibility Repeat Element Alteration (CNV)
Transposon Migration	Hypomethylation DNA	Susceptibility Transposon Migration
Translocation	DNA Methylation and Histone Alterations	Susceptibility Translocation at Break Point
Telomere Length	DNA Methylation Alteration	Alteration in Telomere Length

Environmentally Induced Epigenetic Transgenerational Inheritance of Sperm Epimutations Promote Genetic Mutations

Skinner MK, Guerrero-Bosagna C, Haque M.
Epigenetics 2015; 10:8, 762-771

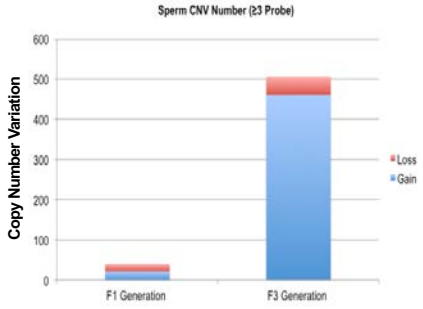
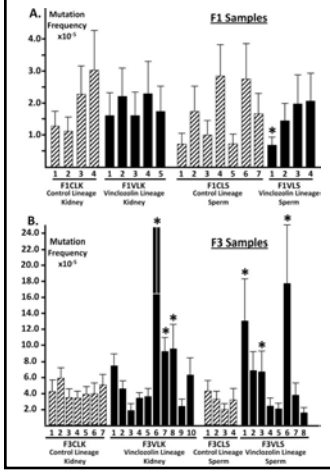


Table 1. (A) Vinclozolin F3 Generation Sperm Genome-wide CNV and Epimutations

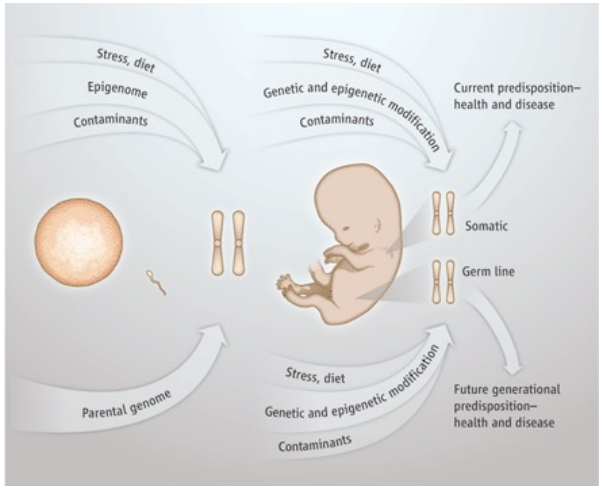
Parameters	F1 Generation Sperm CNV	F3 Generation Sperm CNV	F3 Generation Epimutation Sperm
Number (Single Probe)	540(294 Gain / 246 Loss)	4912(4648 Gain / 264 Loss)	9932
Number (≥3 Probe)	39(21 Gain / 18 Loss)	506(461 Gain / 45 Loss)	191
Mean Size (base)	11,633	12,637	2,131
Mean CpG Density (CpG/100 bp)	1.1	1.0	0.9

Tertiary Epimutations - A Novel Aspect of Epigenetic Transgenerational Inheritance Promoting Genome Instability.

PLoS One. 2016 Dec 19;11(12):e0168038.
McCarrey JR, Lehle JD, Raju SS, Wang Y, Nilsson EE, Skinner MK.



Mutation frequencies in F1 and F3 generation samples.
(A) Mutation frequencies in kidney and sperm samples from F1 generation control- and vinclozolin-lineage animals. There were no statistically significant differences among the mutation frequencies detected in kidney or sperm samples from F1 generation control- and vinclozolin-lineage samples, except for one of the vinclozolin-lineage sperm samples (F1VL– marked with an asterisk) which showed a mutation frequency that was significantly lower than the mean of the F1 generation control-lineage samples ($p = 0.00352$). (B) Mutation frequencies in kidney and sperm samples from F3 generation control- and vinclozolin-lineage animals. A subset of both kidney and sperm samples from F3 vinclozolin-lineage descendants showed mutation frequencies that were not significantly different than the mean of the corresponding F3 generation control-lineage samples, although several of the F3 generation vinclozolin-lineage samples trended higher than the mean of the corresponding F3 generation control-lineage samples. However another subset of both kidney and sperm samples from F3 generation vinclozolin-lineage descendants showed mutation frequencies that were significantly higher than the mean of the corresponding F3 control-lineage samples. These mutation frequencies are marked with astrices, and include those found in the following samples: F3VLK6 ($p = 0.00342$), F3VLK7 ($p = 0.00131$), F3VLK8 ($p = 0.0222$), F3VLS1 ($p = 0.00185$), F3VLS2 ($p = 0.03611$)) and F3VLS6 ($p = 0.00018$). F1 = samples from F1 generation descendants, F3 = samples from F3 generation descendants, CL = samples from control-lineage descendants, VL = samples from vinclozolin-lineage descendants, K = kidney samples, S = sperm samples.

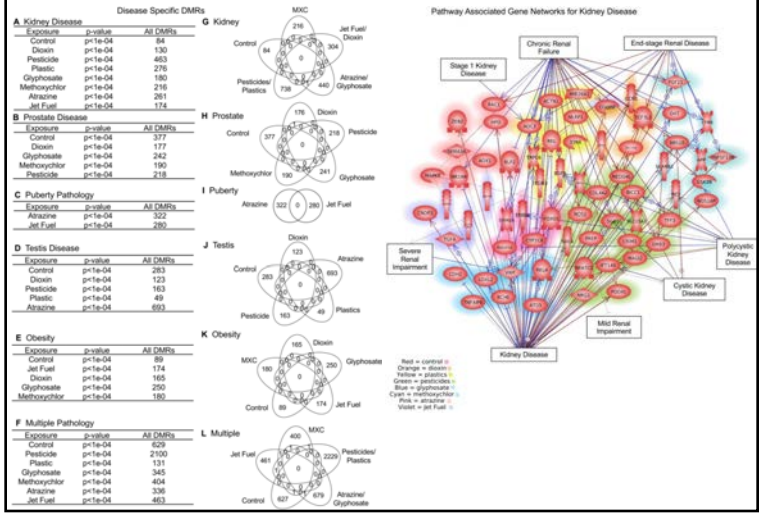


Summary

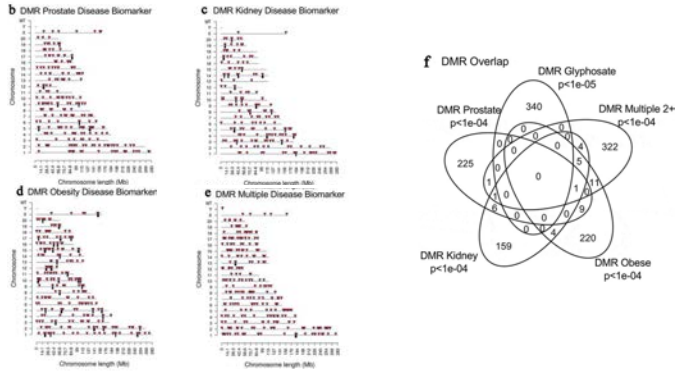
Epigenetic Transgenerational Inheritance
- -Non-Genetic Form Inheritance
- -Toxicology / Environmental
- -Molecular Mechanism for Disease Etiology and DOHAD
- -All Epigenetic Processes Involved and Integrated in Germline
- -Generational Toxicology

Environmental Epigenetics, Disease and Evolution
- -Integration Epigenetics and Genetics Essential Biology
- -Evolution and Disease Etiology Requires Inclusion Epigenetics
- -Doom and Gloom/ Biomarkers & Preventative Medicine

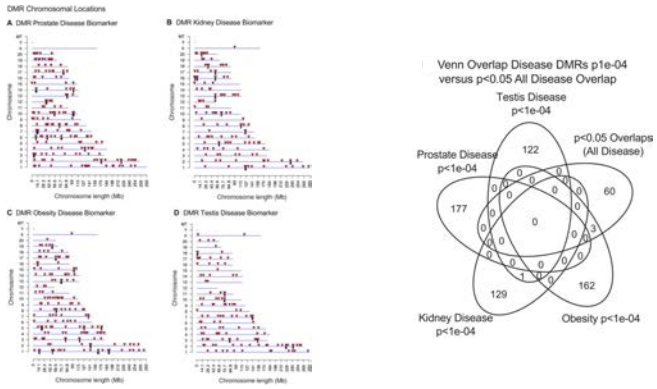
Environmental induced transgenerational inheritance impacts systems epigenetics in disease etiology.
Beck D, Nilsson EE, Ben Maamar M, Skinner MK.
Sci Rep. 2022 Apr 19;12(1):5452.

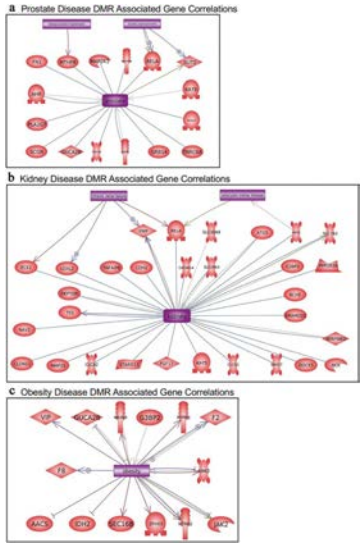


Epigenome-wide association study for glyphosate induced transgenerational sperm DNA methylation and histone retention epigenetic biomarkers for disease.
Ben Maamar M, Beck D, Nilsson EE, Kubsad D, Skinner MK.
Epigenetics. 2020 Dec 9:1-18.



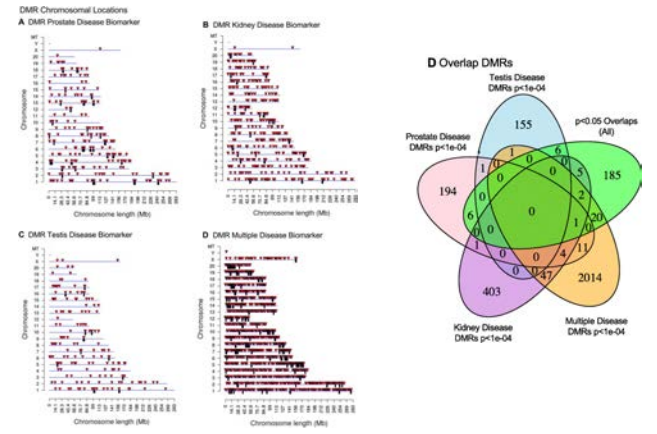
Transgenerational disease specific epigenetic sperm biomarkers after ancestral exposure to dioxin.
Ben Maamar M, Nilsson E, Thorson JLM, Beck D, Skinner MK.
Environ Res. 2021 Jan;192:110279.

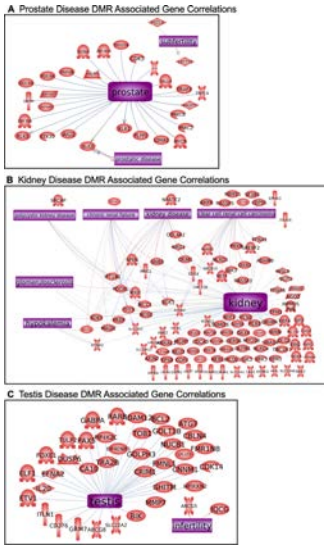




DMR-associated genes within the pathology biomarker DMR set for each individual pathology. The physiologic and pathology process is listed with direct gene links. (a) Prostate disease, (b) kidney disease, and (c) obesity

Epigenome-wide association study for pesticide (Permethrin and DEET) induced DNA methylation epimutation biomarkers for specific transgenerational disease.
Thorson JLM, Beck D, Ben Maamar M, Nilsson EE, Skinner MK.
Environ Health. 2020 Nov 4;19(1):109.

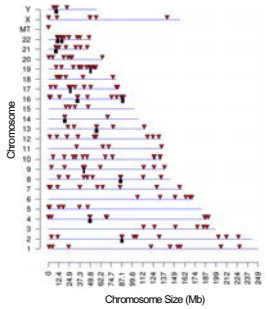




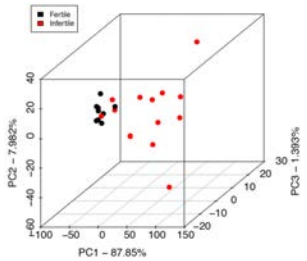
Specific disease DMR associated gene correlation with previously identified disease genes. **a** Epimutation associated prostate disease genes. **b** Epimutation associated kidney disease genes. **c** Epimutation associated testis disease genes. The gene functional category shapes are identified as follows inset in Fig. 8

Sperm DNA Methylation Epimutation Biomarkers for Male Infertility and FSH Therapeutic Responsiveness
Luján S, Caroppo E, Niederberger C, Arce J-C, Sadler-Riggleman I, Beck D, Nilsson E, Skinner MK
Scientific Reports (2019)

Infertility Sperm DMR Chromosomal Locations



PCA Infertility DMR Signature Analysis



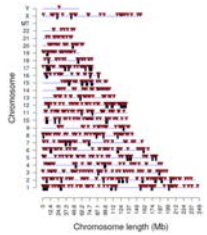
Sperm DNA Methylation Epimutation Biomarker for Paternal Offspring Autism Susceptibility
Garrido N, et al., and Skinner MK
Clinical Epigenetics 2021 13:6 p3-13

DMR Identification

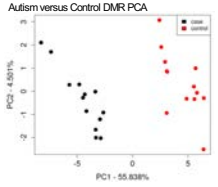
p-value	All Window	Multiple Window
0.001	13271	256
1e-04	3214	31
1e-05	**805**	**6**
1e-06	223	1
1e-07	60	0

Significant windows	1	2	3
Number of DMR	799	3	3

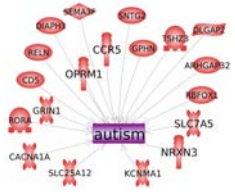
Chromosome Plot



Autism versus Control DMR PCA



DMR Associated Genes and Autism



"Epigenetics and Systems Biology"

Spring 2023 (Odd Years)
Biol 476/576
Schedule/Lecture Outline –

Week 1	January 10 & 12	Systems Biology (History/ Definitions/ Theory)
Week 2	January 17 & 19	Systems Biology (Networks & Emergence)
Week 3	January 24 & 26	Systems Biology (Components: DNA to Phenotype)
Week 4	Jan 31 & Feb 2	Systems Biology (Genomics / Technology)
Week 5	February 7 & 9	Epigenetics (History / Molecular Processes)
Week 6	February 14 & 16	Epigenetics (Molecular Processes & Integration)
Week 7	February 21 & 23	Epigenetics (Genomics and Technology)
Week 8	Feb 28 & March 2	Cell & Developmental Biology
Week 9	March 7 & 9	Epigenetics of Cell & Developmental Biology (& Midterm Exam)
Week 10	**March 13 – 17**	**Spring Break**
Week 11	March 21 & 23	Environmental Impact on Biology
Week 12	March 28 & 30	Environmental Epigenetics
Week 13	April 4 & 6	Disease Etiology
Week 14	April 11 & 13	Epigenetics & Disease Etiology
Week 15	April 18 & 20	Evolutionary Biology & Genetics
Week 16	April 25 & 27	Epigenetics & Evolutionary Biology
Week 17	May 2 & 4	Grant Review/ Study Section Meeting (& Final Exam)