Title: Epigenetics in action: part 3: it runs in the family
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JEAN-BAPTISTE Lamarck, like any good scientist, was trying to develop a scientific theory by collecting data relevant to his hypothesis. He famously recorded that the sons of blacksmiths tended to have larger arm muscles than the sons of weavers (a much less strenuous occupation). In Lamarck's interpretation, the blacksmiths had acquired that physical characteristic (what biologists now call a phenotype) as a result of their work, and their sons had inherited their fathers' physique. Our modern interpretation is different. We recognize that a man whose genes tended to endow him with the ability to develop large muscles would be at an advantage in a trade such as blacksmithing; the blacksmith's sons may have inherited this genetic tendency toward chunky biceps. Finally, we would acknowledge that the children of a blacksmith might have been pumping more iron, so to speak, from an early age than those of a weaver.

Charles Darwin, the colossus of nineteenth-century biology, certainly overshadows Lamarck's endeavors. Darwin's model of the evolution of species via natural selection has been the single most powerful conceptual framework in biological sciences. Its power became even greater once married to Gregor Mendel's work on inheritance and then to our molecular understanding of DNA as the raw material of inheritance.

It would be a mistake to look back on Lamarck and only mock. But given the overwhelming body of data arguing against Lamarckian inheritance, there's been very little reason for individual scientists to further investigate acquired characteristics. Moreover, some scientists may have shied away to avoid scandal. One of the most notorious cases of scientific fraud is that of Austrian biologist Paul Kammerer, who worked in the first half of the twentieth century. He claimed to have demonstrated the inheritance of acquired characteristics in the midwife toad. When one of his experimental subjects was found to have had India ink injected into the pads of its feet, Kammerer was accused of fabricating his results. Kammerer killed himself soon after, insisting in his suicide note that he was not the perpetrator of the fraud. The scandal further tainted an already controversial field.

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Yet we now know that there are epigenetic, or molecular, modifications that, though they don't change the DNA sequence of a gene, do alter how the gene is expressed. Those modifications, be they adding a methyl group to DNA or altering adjacent histone proteins, occur at specific genes in response to the state of given cell [see "Part 2: Turn-ons and Turn-offs," May 2012]. Epigenetic modifications of gene expression can be transmitted from a parent cell to a daughter cell, as happens normally in fetal development when cells with the same complete genome become differently specialized, accounting for why there are no teeth in your eyeballs. If a similar mechanism transmitted an environmentally-induced epigenetic modification from parent to child, we would have a sort of Lamarckian inheritance, one that could have serious repercussions for the future of human health.

SOME OF THE STRONGEST EVIDENCE for transgenerational inheritance (that is, the phenomenon of transmission of an acquired characteristic) in humans comes from the survivors of the Dutch Hunger Winter [see "Part 1: Deciphering the Link between Nature and Nurture," April 2012]. Pregnant women who suffered malnutrition during the first three months of pregnancy gave birth to babies who were normal weight early in life but in adulthood were at higher risk of obesity and other disorders. Bizarrely, when women in that next generation became mothers themselves, their firstborn children tended to be heavier than those of control groups. This seems like a good example of Lamarckian inheritance, but has it been caused by an epigenetic mechanism? Maybe, but we shouldn't ignore other potential explanations.

For example, there could be an unidentified consequence of early malnutrition that affects conditions in the womb. It's also important to remember that a human egg is large. It contains a nucleus that is relatively small in volume compared with the surrounding cytoplasm: imagine a grape inside a tangerine. The cytoplasm carries out a lot of functions when an egg gets fertilized. Perhaps something occurs during early developmental programming in malnourished female embryos that ultimately results in unusual cytoplasm of their eggs. That might sound unlikely, but egg production in female mammals is actually initiated early in their own embryonic development. The earliest stages of zygote development rely to a large extent on the cytoplasm from the egg. An abnormality in the cytoplasm could stimulate an unusual growth pattern in the fetus. This would result in a transgenerational effect, but not through the inheritance of an epigenetic modification.

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It would help us to understand if epigenetics plays a role in acquired inheritance if we could study a less complicated human situation. So let's hear it for fathers. Because men don't get pregnant, they can't contribute to the developmental environment of the fetus, nor do they contribute much cytoplasm to the zygote. (Sperm are almost all nucleus, little DNA bullets with tails attached.) So if we see transgenerational inheritance from father to child, an epigenetic mechanism would be an attractive candidate for explaining an acquired characteristic.

THERE IS A GEOGRAPHICALLY ISOLATED region in Northern Sweden called Overkalix. In the late nineteenth and early twentieth centuries that region had periods of terrible food shortages (caused by failed harvests, military actions, and transport inadequacies) interspersed with periods of great plenty.

Scientists have studied the mortality patterns for male descendants of men who were alive during those periods. In particular, they analyzed food intake during a stage in childhood known as the slow growth period (SGP). All other factors being equal, children grow slowest in the years leading up to puberty, a completely normal phenomenon, seen in most populations. Using historical records, the researchers deduced that if food was scarce during a father's SGP, his sons were at decreased risk of dying through cardiovascular disease (such as stroke, high blood pressure, or coronary artery disease). If, on the other hand, a man had access to a surfeit of food during the SGP, his grandsons were at increased risk of dying as a consequence of diabetes.

The sons and grandsons had an altered phenotype (a change in the risk of death through cardiovascular disease or diabetes) in response to an environmental challenge they themselves had never experienced. Therefore, it seems reasonable to hypothesize that the transgenerational consequences of food availability in the grandparental or parental generation were mediated via epigenetics. These data are particularly striking when you consider that the original nutritional effect happened when the boys were prepubescent and so had not even begun to produce sperm. Even so, they were able to pass an effect on to their sons and grandsons.

Of course, there are risks involved in relying on old death records, and extrapolating backwards through historical data. Additionally, some of the observed effects were not terribly large--a frequent problem when working with human populations. There is always the risk that we draw inappropriate conclusions from our data, much as we believe Lamarck did with his studies on the families of blacksmiths.

IF THE EVIDENCE from men or women leaves room for uncertainty, can we get help from other species? The laboratory-bred agouti mouse, which carries the agouti gene, offers some good solutions. Australian geneticist Emma Whitelaw has shown that the agouti's coat color is controlled by an epigenetic mechanism, specifically methylation of a DNA sequence called a retrotransposon, which is next to the agouti gene. Yellow or mottled fur occurred when the retrotransposon was unmethylated, whereas dark fur meant it was methylated. Whitelaw decided to investigate whether the mouse's variable coat color could be inherited. If so, it would show that it's not only DNA that gets transmitted from parent to offspring, but also epigenetic modifications to the genome.

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When Whitelaw allowed female agouti mice to breed, she found that if the mother had yellow fur (unmethylated

retrotransposon), all of her offspring also had either yellow fur or slightly mottled fur; she never had offspring that developed dark fur. By contrast, if the mother's sequence was heavily methylated, resulting in her having dark fur, one in five of her offspring also had dark fur. If both grandmother and mother had dark fur, then the effect was even more pronounced. About a third of the final generation had dark fur, compared with the one in five in the second generation.

Because Whitelaw was working on inbred mice, she was able to perform this experiment multiple times and generate hundreds of genetically identical offspring. Statistical tests showed that the phenotypic differences between the genetically identical groups were highly significant. In other words, it was very unlikely that the effects occurred by chance.

Whitelaw and her laboratory group had to be careful to control for the effect of intrauterine nutrition and environment in their experiments. In one of those kinds of experiments that simply aren't possible in humans, they transferred fertilized eggs obtained from yellow mothers and implanted them into dark females, and vice versa. In every case, the distribution of coat patterns in the offspring was the same as was to be expected from the egg donor--that is, the biological mother--rather than the surrogate. Also, by using complex breeding schemes, they demonstrated that the inheritance of the coat pattern was not due to the cytoplasm in the egg. Taken together, the most straightforward interpretation of these data is that epigenetic inheritance has taken place. In other words, an epigenetic modification was transferred along with the genetic code.

The transfer of the phenotype from one generation to the next wasn't perfect--not all the offspring looked exactly the same as their mother. That implies that the DNA methylation that controls the expression of the agouti phenotype wasn't entirely stable down the generations. This is quite analogous to the effects we see in suspected cases of human transgenerational inheritance, such as the Dutch Hunger Winter. If we look at a large enough number of people in our study group we can detect differences in birth weight between various groups, but we can't make absolute predictions about a single individual.

There is also an unusual gender-specific phenomenon in the agouti strain. Although coat pattern showed a clear transgenerational effect when it was passed on from mother to pup, no such effect was seen between father and pup. It didn't matter if a male mouse was yellow, lightly mottled, or dark. When he fathered a litter, there were likely to be all the different patterns of color in his offspring.

Other examples of epigenetic inheritance are transmitted from both male and female mice, such as the variably kinked tails in genetically identical mice with the [Axin.sup.Fu] (Axin fused) gene (caused by variable methylation of a retrotransposon in the gene). These model systems have been tremendously useful in demonstrating that transgenerational inheritance of a nongenetic phenotype does actually occur, and that this takes place via epigenetic modifications. This is truly revolutionary. It confirms that in some very specific situations Lamarckian inheritance is taking place, and we have a handle on the molecular mechanism behind it. But the agouti and kinked-tail phenotypes in mice both rely on the presence of specific retrotransposons in the genome. Are these special cases, or is there a more general effect in play? Let's turn to something that has a bit more immediate relevance for us all. Food.

AS WE ALL KNOW, an obesity epidemic is developing. It's spreading worldwide, advancing at a particularly fast rate in the more industrialized societies. Figures for the United Kingdom population in 2007 indicate that about two out of every three adults is overweight (body mass index of 25 or over) or obese (body mass index of 30 or over). The situation is even worse in the United States. Obesity is associated with a wide range of health problems, including cardiovascular disease and type 2 diabetes. Obese individuals over the age of forty will die, on average, six to seven years earlier than non-obese people.

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The data from the Dutch Hunger Winter and other famines support the idea that poor nutrition can have epigenetic effects on later generations. The data from the Overkalix cohort, although more difficult to interpret, suggested that excess consumption at key points in a boy's life can have adverse consequences for later generations. What effects will the obesity epidemic have for children and grandchildren? As we don't really want to wait forty years to work this out, scientists are again turning to animal models to try to gain some useful insights.

In 2010, two papers were published that should give us pause. In both cases, the researchers overfed male animals and then

monitored the effects on their offspring. By restricting their experiments to males, they didn't need to worry about the intrauterine and cytoplasmic complications that cause such (metaphorical) headaches when studying females.

One of the studies used a breed of rat called Sprague-Dawley. This is an albino rat with a chilled-out temperament that makes it easy to keep and handle. In the experiments, male Sprague-Dawleys were given a high-fat diet, and allowed to mate with females that had been fed an ordinary diet. The overfed males were overweight (hardly a surprise), had a high percentage of fat to muscle, and had many of the symptoms found in type 2 diabetes in humans. were weight, they too the diabetes-type abnormalities the genes control metabolism and mammals turn fuel were regulated these off-spring. For reasons that aren't understood, the daughters particularly showed this effect.

The other study, completely independent of first, used an inbred mouse strain. Male mice were fed a diet that was abnormally low in protein but with an increased percentage of sugar to make up for it. The males were mated to females on a normal diet. The researchers examined the expression of genes in the liver (the body's major organ when it comes to metabolism) in three-week-old pups from these pairings. Analyzing large numbers of mouse pups, they found that the regulation of many of the genes involved in metabolism was abnormal in the offspring of the males that had been fed the modified diet. They also found changes in epigenetic modifications in the liver cells of these pups.

Both these studies show us that, at least in rodents, a father's diet can directly influence the epigenetic modifications, gene expression, and health of his off spring. And not because of environment: this isn't like the human example of kids getting fat because their Dad only ever feeds them supersized portions of burgers and chips. And it can't have been due to genetic change, say, diet-induced mutations--they just don't happen that fast. So the most likely explanation is that diet induces epigenetic effects that can be transmitted from father to child.

IF YOU LOOK at all the data in its entirety--from humans to rodents, from famine to feast--a quite worrisome pattern emerges. Maybe the old saw "we are what we eat" doesn't go far enough. Maybe we're also what our parents ate and what their parents ate] before them. This might make us wonder if there is any point to following advice on healthy living. If we are all victims of epigenetic determinism, this would suggest that our dice have already been rolled, and we are just at the mercy of our ancestors' methylation patterns. But this is far too simplistic a model. Overwhelming amounts of data show that the health advice issued by government agencies and charities-eating a healthy diet rich in fruit and vegetables, getting off the sofa, not smoking--is completely sound. We are complex organisms, influenced by our genome, our epigenome, and our environment. Even in the inbred agouti mice, kept under standardized conditions, researchers couldn't predict exactly how yellow or how fat an individual mouse in a newborn litter would become. Why not do everything that we can to improve our chances of a healthy and long life? And if we are planning to have children, don't we want to do whatever we can to nudge them that bit closer to good health?

There will always be things we can't control, of course. One of the best-documented examples of an environmental factor that has epigenetic consequences, lasting at least tour generations, is an environmental toxin. Vinclozolin is a fungicide that tends to be used particularly frequently in the wine industry. If it gets into mammals, it is converted into a compound that binds to androgen receptors. These are the receptors that bind testosterone, the male hormone that is vital for sexual development, sperm production, and a host of other effects in males. When vinclozolin binds to androgen receptors, it prevents testosterone from transmitting its usual signals to the cells, and so blocks the normal effects of the hormone.

If vinclozolin is given to pregnant rats at the time when the testes are developing in the embryos, the male offspring are born with testicular defects and have reduced fertility. The same effect is found for the next three generations. About 90 percent of the male rats are affected, which is far too high a percentage to be caused by classic DNA mutation. Even the highest known rates of mutation, at particularly sensitive regions of the genome, are at least tenfold less frequent than this. In these rat experiments, only one generation was exposed to vinclozolin, yet the effect lasted for at least four generations. Given the male transmission pattern, it is likely this is another example of an epigenetic inheritance mechanism. The same research group that initially discovered vinclo zolin's "Lamarckian" effects has since identified regions of the genome where vinclozolin treatment leads to unusual DNA methylation patterns.

The rats in the studies described above were treated with high doses of vinclozolin, much larger than humans are believed to encounter in the environment. Nonetheless, effects such as these are one of the reasons why some authorities are beginning to investigate whether artificial hormones and hormone disrupters in the environment (ranging from chemicals excreted by women on the contraceptive pill to certain pesticides) have the potential to cause subtle, but potentially transgenerational effects in the human population.

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Nessa Carey was employed at the Metropolitan Police Forensic Science Lab in London before earning a degree in immunology and a doctorate in virology. She then followed the academic route of post-doc and university lecturer, becoming a senior lecturer in Molecular Biology at Imperial College, London, where she led a research team investigating a genetic disorder that gets worse as it passes down through the generations in an affected family. Subsequently, for the past ten years, she has worked in the biotech and pharmaceutical industry. Carey lives in Bedfordshire, England (www.nessacarey.co.uk).

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