Genomic Imprinting

Genomic imprinting is one of the most surprising and mysterious findings in evolutionary biology. Genes sometimes carry an imprint of their parental origin and have different expression patterns when they come from a mother or a father. For example, the copy of the mouse insulinlike growth factor Igf2 inherited from the father is turned on, but the copy inherited from the mother is totally suppressed. These parental imprints are erased and reset each generation. Thus, male offspring will pass on active copies of Igf2 to its children whereas female offspring will pass on inactive copies. The memory of whether these genes came from the grandmother or the grandfather is erased, and a new imprint reflecting the offspring's gender is reapplied during sperm or egg production. The mechanisms controlling imprinting are now well understood, but the evolution of this strange control of gene expression remains a matter of contention.

Imprinting was first discovered by nuclear transfer studies in mice carried out in the 1980s. Micromanipulation was used to move haploid female or male pronuclei between recently fertilized eggs (Figure 1). A normal fertilized egg contains one female and one male pronucleus (1M:1P). If the male pronucleus is removed and replaced with a female pronucleus, the resulting gynogenetic egg contains two female pronuclei (2M:0P). The reverse manipulation results in an androgenetic egg containing two male pronuclei (0M:2P). Gynogenetic and androgenetic eggs initiate development, implant normally, but then fail to thrive, and all eventually die: this despite the fact that both kinds of egg contain a full set of genes. In contrast, eggs reconstituted with the normal pattern of one female and one male pronucleus develop normally to term.

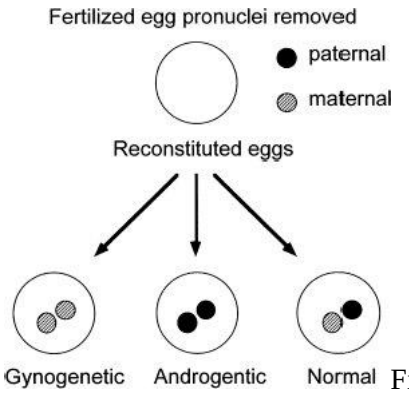


Figure 1. Transplantation Experiments.
The maternal and paternal pronuclei were removed from fertilized mouse eggs, then replaced to create gynogenetic (2M:0P), androgenetic (0M:2P) and normal (1M:1P) diploid eggs. Only the normal eggs developed normally to term.
Courtesy of Andrew Pomiankowski.

Imprinting is also evident at the level of chromosome regions. A dramatic example in humans is deletion of the q11–13 region of chromosome 15. Maternal inheritance (i.e., inheritance from

the mother) of the deletion causes Angelman syndrome (hyperactivity, inappropriate laughter, repetitive movements), whereas paternal inheritance causes Prader-Willi syndrome (hypotonia, short stature, small gonads). These different clinical syndromes are not caused by the deletion of different genes. The same genes are missing in both cases, but these deletions have very different effects when inherited from the mother or the father.

These parent-of-origin effects were pinned down to particular genetic loci during the 1990s. At the last count, over forty genes have been shown to be imprinted in mice and humans. One of the best understood cases is the mouse Igf2 gene. Denise Barlow and colleagues demonstrated imprinting by investigating a null allele of Igf2 (i.e. a deletion of this gene). The null allele has no detectable phenotype when maternally inherited, but gives rise to mice that are considerably smaller than their littermates when paternally inherited. As the maternal copy of Igf2 is silent, inheritance of the null allele from the mother has no effect. In contrast, the paternal copy of Igf2 is usually active. Thus, paternal inheritance of the null allele causes a reduction in gene expression and in this case leads to a loss in body size.

## Mechanism of Imprinting

To understand imprinting at a mechanistic level, we need to explain a number of key features. Imprinting involves generation of distinct maternal and paternal marks on the DNA that alter gene expression, maintenance of parental marks through somatic cell division, and erasure of marks during gametogenesis, along with the reapplication of novel marks to reflect current gender. Thus, imprints need to be permanent within a generation but reversible between generations.

Silencing of imprinted genes is achieved by changes in DNA structure, not by changes in DNA sequence. Most imprinted genes show differences between the maternal and paternal copies in histone deacetylation and DNA methylation. Histones are DNA-binding proteins that form nucleosomes, the basic structure of chromatin. When histones are deacetylated, the nucleosomes condense into a tightly bound structure. In addition, upstream of most imprinted genes are DNA sequences that are relatively rich in the dinucleotide CpG (cytosine followed by guanine). The cytosine base in these CpG dinucleotides can be altered by the addition of a methyl group ($CH_3$). Like histone deacetylation, methylation is associated with chromosomal condensation. These changes to the DNA structure are capable of causing differential gene expression in a number of ways. The main effect of condensation is to block access to DNA sequences that act as promoters (or repressors), thereby turning off (or on) gene transcription.

Once established, patterns of deacetylation and methylation are maintained through cell division. Most is known about the maintenance of methylation. When a DNA molecule is replicated, the two daughter chromosomes are methylated on one strand but not the other (Figure 2). A set of Dnmt methylation enzymes recognizes this hemimethylated state and reestablishes methylation on both strands of the DNA. Nonmethylated DNA is not recognized

by the methylation enzymes and remains nonmethylated after replication. The net effect is that methylated and nonmethylated alleles can coexist in the same cell, and their states are stably maintained through cell divisions. This allows maternal and paternal alleles to retain their imprinting status throughout growth and development.

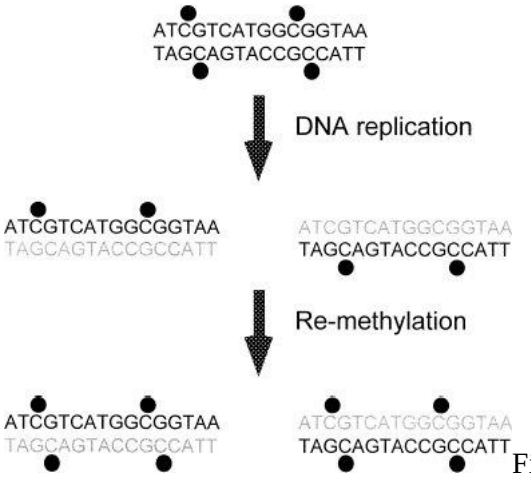


Figure 2. Replication of DNA Methylation. Methylation of cytosine residues (blackspots) in CpG dinucleotides in the DNA sequence. This occurs on the plus strand (top, running left to right) and the complementary minus strand (bottom, running right to left). After DNA replication, the new strand is not methylated. A set of enzymes recognize hemimethylated sites and remethylate the cytosine base of the new strand. This allows the pattern of methylated sites and remethylate the cytosine base of the new strand. This allows the pattern of methylation to be perpetuated through cell division. A = adenine. C = cytosine. G = guanine. T = thymine.
Courtesy of Andrew Pomiankowski.

Imprinting is reset in the germ line of both sexes. Genome-wide demethylation occurs soon after embryonic establishment of the germ line. This removes the preexisting maternal or paternal marks. As the germ cells start to differentiate into sperm and eggs, a process of de novo methylation begins. Unfortunately, little is known about the enzymes involved, how sites are identified for deacetylation and methylation, or how sex-specific imprints are established.

## Conflict Hypothesis

The evolution of genomic imprinting at first appears paradoxical. There seems no obvious benefit to turning off gene expression from one gene, especially as this predisposes the individual to deleterious somatic mutations that are usually masked by the presence of the second copy. The lack of any straightforward benefit from imprinting has led to many ill thought out and poorly supported hypotheses. For instance, no mammal is known to develop asexually through parthenogenesis (i.e., from an unfertilized egg). This is ruled out by imprinting, as the paternal genome with its particular pattern of imprints is necessary for normal development. While the absence of parthenogenesis is a consequence of imprinting, it hardly

seems a plausible reason for the evolution of around 100 imprinted genes in the mammalian genome.

The rejection of inappropriate hypotheses has become easier since David Haig (2000) proposed a general mechanism for the evolution of imprinting. This is known as the conflict hypothesis and arises from a simple observation about relatedness. Haig's hypothesis has the virtue that it potentially can explain many of the phenomena associated with imprinting. However, as more data have been uncovered, it has become less clear whether conflict is the main selective force or just one of many.

The reason for conflict between maternal and paternal copies of the same gene within an individual arises from differences in relatedness. This is easily shown by considering the offspring of a single female (Figure 3). As a result of Mendelian inheritance, a maternally derived gene has a 50 percent chance of being present in other progeny of the same mother. This probability is constant and independent of the mating system (e.g., monogamous, polygamous). In contrast, the relatedness of paternally inherited genes depends on the degree of multiple mating. If the mother is monogamous (i.e., she mates only with a single male throughout her reproductive life), all her offspring will have a 50 percent chance of sharing identical paternal genes. But in most mammal species females mate with multiple males. Among siblings, paternally inherited genes are often from different fathers, both within and between litters. Thus, average relatedness of paternally inherited genes is typically considerably less than 50 percent.

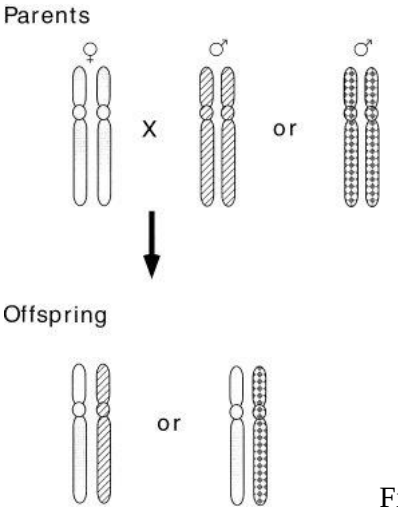


Figure 3. Relatedness Between Maternal and Paternal Genes in Offspring When a Female Mates with Two Males.

Offspring have a 50 percent probability of sharing maternal genes, but the probability is much lower for paternal genes, as these may originate from different fathers. This creates a relatedness asymmetry between maternal and paternal genes, which increases with the degree of multiple paternity.

Courtesy of Andrew Pomiankowski.

This difference in relatedness has a profound effect on mother–offspring interactions. Given that a mother is equally related to all her current and future offspring, she benefits from distributing her resources relatively evenly within a litter and retaining resources for the future. In offspring, maternally inherited genes are under similar selection, as they too are present in all current and future offspring. However, paternally inherited genes have much less interest in other offspring, which are far less likely to carry related genes. We thus expect paternally inherited genes to be far more demanding of resources from the mother, even if their own gain is to the detriment of others within the same brood. The paternally inherited genes also care less if the current offspring demand decreases the mother's survival chances as future offspring are unlikely to be fathered by the same male.

It is easiest to see the evolutionary consequence of selection on maternal and paternal genes in the extreme case in which all offspring have different fathers. Consider a gene coding for an embryonic growth factor. Mutants that increase paternal gene expression are favored by selection, as an embryo producing more growth factor receives more resources from the mother, and thus enjoys better survival chances. This reduces the number and survival of other offspring produced by the same mother. However, this does not affect selection on the mutant, as the paternal gene is not present in other progeny. Once increases in paternal gene expression are established, there is counterselection in favor of less demand from the maternal gene. Mutants that marginally reduce maternal gene expression are selected despite any loss in fitness because they benefit other offspring that have a 50 percent probability of carrying the mutant. These counteracting forces lead to a coevolutionary “arms race,” in which increases in paternal copy demand are matched by decreases in maternal copy demand. The process continues until the maternal copy is silenced and growth factor is produced only by the paternally inherited gene.

## Evidence for the Conflict Hypothesis

Some gross distortions of parental contributions to embryos fit the predictions of the conflict hypothesis. As mentioned above, gynogenetic (2M:0P) and androgenetic (0M:2P) embryos fail to develop, but for different reasons. Gynogenetic embryos abort because the placenta fails to develop properly, whereas androgenetic embryos abort because of disproportionately large growth of the placenta. These observations suggest that maternal genes favor low-growth demand and paternal genes favor high-growth demand.

The most stunning evidence in favor of the hypothesis comes from the mouse insulinlike growth factor Igf2. This embryonic growth factor is silent when maternally inherited and active when paternally inherited. However, its receptor gene, Igf2r, shows the reverse pattern, being active when maternally inherited and silent when paternally inherited. The Igf2r receptor binds to the Igf2 protein, inactivates its signaling function, and leads to its degradation. Thus, the Igf2r gene acts as a growth repressor, and, as predicted by the conflict hypothesis, it is a maternally active and paternally inactive gene.

Data from a number of imprinted genes have accumulated during the last decade and now allow a more general test of the conflict hypothesis. The best data come from individuals with uniparental disomies (where both chromosomes are inherited from one parent) and mouse gene knockouts (genetically engineered microdeletions). Most maternal disomies are growth retarding, as predicted by the conflict hypothesis. However, nearly all paternal disomies are also growth retarding, only one being unambiguously growth enhancing. Thus, overall, the evidence from disomies does not support the conflict hypothesis. But it is difficult to put too much weight on this finding, as the effect of disomy per se is probably too great, and so obscures most differences between maternal and paternal origins of the disomy.

Knockouts of a number of imprinted genes show the predicted pattern of paternal knockout embryo growth suppression and maternal knockout embryo growth enhancement (e.g., H19, Grf1, and Gnas). However, not all data fit so nicely. Several genes have no or ambiguous effects (e.g., Ins, Ins2, and Snrpn), and some have the reverse effects on embryo growth (e.g., Mash2). In addition, some imprinted genes have effects that are hard to interpret as part of maternal-embryotic growth regulation. A number of imprinted genes are important in brain development. For example, Peg1 and Peg3 are both imprinted genes expressed in the brain that are paternally active. When the paternal copy is deleted, female behavior toward offspring is abnormal (male behavior is normal).

At first glance, these findings suggest that maternal-paternal conflict is not the only force governing the evolution of genomic imprinting. But knockouts are only a crude test of the theory, because they reduce gene expression to zero. It has been suggested that more minor changes in expression might still conform to the conflict hypothesis predictions. Although these are clever post hoc explanations, what is really needed is experiments, and these remain to be attempted. Another area where more data are needed is from species with lower or higher rates of multiple mating. At the moment, investigation has centered on mice and humans. Some imprinting differences have been identified (e.g., Igf2r is not imprinted in humans), but it is difficult to make sense of these. What is needed are comparisons between closely related species that differ in mating system.

### X-linked Imprinting

The phenomenon of X-linked imprinting suggests an intriguing alternative to the conflict hypothesis. Two examples of imprinting are known from comparisons of individuals with a single X chromosome that is either maternally ($X^mO$) or paternally ($X^pO$) derived. These XO individuals develop as females. In mice, the imprinted X affects embryonic growth rate. $X^pO$ embryos are smaller than normal $X^mX^p$ females, which in turn are smaller than $X^mO$ embryos. This pattern of growth enhancement by the maternal X is contrary to the conflict hypothesis. In humans, XO females suffer from Turner's syndrome. In this case, $X^pO$ children have better

social cognitive skills than $X^mO$ children. It is not obvious how this behavioral difference can be accounted for by the conflict hypothesis.

An alternative explanation for X-linked imprinting derives from the pattern of inheritance of the X chromosome (Figure 4). The maternal X is transmitted equally to female and male offspring, whereas the paternal X is only passed to female offspring. This inheritance asymmetry allows X-linked imprinted genes to have sex specific effects. In females, expression of X-linked genes is the average of $X^m$ and $X^p$ contributions (in female mammals, X-linked genes undergo dosage compensation). In contrast, male gene expression is just from the maternal copy. Thus, if the paternal copy is silenced by imprinting, it reduces female gene expression but has no effect on males, and if the maternal copy is silenced by imprinting, it predominantly reduces male gene expression. This allows us to make sense of the two examples of X-linked imprinting. In mice, the X-linked imprint is paternally silenced and leads to faster growth in male offspring (which carry the active maternal X). In humans, the X-linked imprint is maternally silenced and leads to better social cognitive skills in female offspring (who carry the active paternal X and the inactive maternal X). It is easy to imagine that these sex differences are the result of different selection pressures on the two sexes.

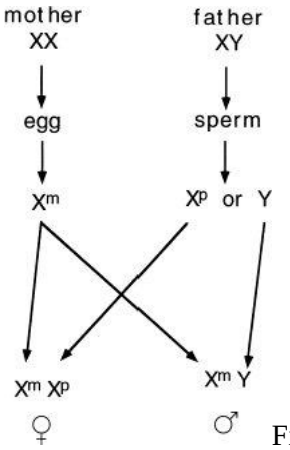


Figure 4. Sex Specific Inheritance of X-Linked Imprints.
The maternal $X^m$ is inherited by both sexes, whereas the paternal $X^p$ is passed only to daughters. Journal of Theoretical Biology. Academic Press, 1999.

These findings are interesting, as they provide a concrete alternative explanation to the conflict hypothesis. Imprinting on the X appears to be the result of selection for sex-specific gene expression rather than conflict. The one caveat here is that the X-linked imprinted genes have not yet been mapped, so there may be some surprises when this happens. The sex-specific explanation cannot apply to imprinting on autosomes, as these are inherited equally by both sexes (except in some insects with haplodiploid patterns of inheritance). But the lack of evidence that conflict has shaped imprinted X-linked genes does suggest that conflict is not the only selective pressure and alternative forces may explain some autosomal imprints.

## Conclusion

In this article, we have concentrated on explaining genomic imprinting in mammals. This is because mammalian imprinting is well studied, and related vertebrates (birds, fish, and reptiles) appear to lack imprinting at the level of individual genes. However, imprinting is found in other groups. In particular, it has evolved and controls individual gene expression in the flowering plants, where it affects parental investment in seeds. The logic of the conflict hypothesis may apply here as well. There are also several examples of genome imprinting in insects, amphibia, and fish in which the whole maternal or paternal genomes are turned off or ejected from cells. The evolution and mechanistic basis of these phenomena are probably unrelated to individual gene imprinting in mammals and plants, but little is fully understood.

The next few years promise to be exciting for research in genomic imprinting. Many more imprinted genes will be characterized, and a variety of nonstandard species (i.e., not humans or mice) are starting to be investigated. This information will be important in gaining a better understanding the selective forces maintaining imprinting. It will then be possible to undertake better tests of the conflict hypothesis and other evolutionary explanations of imprinting.

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