2

The evolution of parental imprinting: a review of hypotheses

DAVID HAIG AND ROBERT TRIVERS

Introduction

Parental imprinting has been suggested to be an adaptation for preventing parthenogenetic development (Solter, 1988), an expression of genetic conflicts between maternal and paternal genomes (Haig & Westoby, 1989), an outcome of dominance modification (Sapienza, 1989), a means to restrain the growth of the placenta (Hall, 1990), a mechanism of growth factor regulation (Cattanach, 1991), a consequence of host defense mechanisms (Barlow, 1993), and a device to protect females against malignant germ-cell tumors (Varmuza & Mann, 1994). Are these different hypotheses alternative answers to the same question? If so, does the evidence allow us to choose among them?

The purpose of this paper is to review ideas about the adaptive function (or lack of function) of imprinting. We are not impartial commentators and will defend the genetic-conflict hypothesis against other functional hypotheses. Our discussion will often make a distinction between functions of a DNA sequence and side-effects of the sequence. An *effect* of a sequence is a *function* if it has positively contributed to the spread and present frequency of the sequence. All other effects of the sequence are *side-effects*. The italicized terms are defined with greater precision in the Appendix.

The genetic-conflict hypothesis

Genes that are expressed in an individual's soma do not leave direct descendants but are selected to promote the transmission of copies of themselves via the individual's germline. By extension, a gene in the soma of one individual can be selected to promote the transmission of copies of itself in the germlines of other individuals. Hamilton (1964) showed that a gene from one individual will value the reproduction of another individual in proportion to the probability that this

individual carries a copy of the gene by direct descent from a common ancestor. The maternal and paternal genes of the first individual may have different probabilities of being present in the second individual. These different values become a source of conflict when the expression of genes in the first individual benefits the first individual at cost to the second, or the second at cost to the first. In such cases, maternal and paternal alleles can be selected to have different patterns of expression (Haig, 1992a).

One situation in which maternal and paternal alleles come into conflict arises from the interaction between mothers and their offspring. Increased maternal investment in one offspring results, on average, in fewer resources available for other offspring of the same mother (including potential future offspring). Therefore, genes that are expressed in offspring will have been selected to make lesser nutritional demands on mothers when the genes are maternally derived than when the genes are paternally derived. This is because the paternal genes of an offspring will value the mother's other offspring less highly than do the maternal genes of the same offspring, whenever there is some possibility that the mother produces offspring by more than one father (Haig, 1992b, 1993a; Haig & Graham, 1991; Haig & Westoby, 1989, 1991).

Other situations can be imagined in which interactions occur between individuals that differ in their degree of relatedness through the maternal and paternal line. For example, many mammals form matrilineal associations in which daughters breed near their mothers while breeding males are outsiders that temporarily enter the group. Altruistic behaviors that benefit kin (e.g. warning cries, support in fights) would be more strongly favored by maternal genes than by paternal genes. Similarly, neighboring plants may be more closely related through the maternal line than the paternal line if pollen has a greater dispersal distance than seeds. The maximum difference between maternal and paternal relatedness is progressively diminished for relatives at greater and greater remove, and the opportunities for conflict within the genome are similarly reduced.

When there is conflict within the genome, the concept of an adaptation of the organism is ill-defined because a given phenotype can favor the transmission of some of the organism's genes but not others. Thus, an aspect of phenotype that is an effect of more than one gene can be a function of one but a side-effect of another. Sapienza (1989) has emphasized the distinction between imprinted genes and imprinting genes. The parent-specific expression of an imprinted gene is an effect of imprinting genes (if these exist) but is also an effect of the gene that is imprinted if there are sequence elements of the imprinted gene (e.g. an 'imprinting box') that make it susceptible to the action of imprinting genes. This is because one can imagine variant alleles at the imprinted locus that are not subject to modification by the imprinting genes. The genetic-conflict hypothesis proposes

that imprinting is a *function* of imprinted genes, but is neutral about whether or not imprinting is a function, rather than a side-effect, of imprinting genes.

The hypothesis has little to say about the mechanisms of imprinting. Rather, it predicts that certain kinds of genes will be imprinted (and not others) in certain kinds of organisms (and not others). These predictions are independent of the precise mechanism of imprinting. If there is only one conceivable way in which imprinting can be achieved, all imprinted genes should be imprinted in the same manner, but, if there are many possible mechanisms of imprinting, the particular mechanism adopted may be idiosyncratic for each imprinted gene.

Taxonomic distribution

The genetic-conflict hypothesis predicts that imprinting will have an important role during embryonic development in viviparous taxa, but will be less important in oviparous taxa. Consistent with these predictions, imprinting has significant effects on development in flowering plants and mammals, but is thought to be less important (or absent) in most non-mammalian vertebrates (Haig & Westoby, 1989). Major developmental effects of imprinting appear to be absent in two well-studied oviparous organisms. In *Caenorhabditis elegans*, maternal and paternal uniparental disomy of each chromosome pair is compatible with normal development (Haack & Hodgkin, 1991); similar data exist for *Drosophila melanogaster* (Lindsley & Grell, 1969). Thus, the taxonomic distribution of parental imprinting provides qualified support for the genetic-conflict hypothesis.

Coccoid scale insects and sciarid flies are an exception to the generalization that imprinting is absent from oviparous taxa. In these insects, paternal chromosomes are eliminated during spermatogenesis so that every sperm carries maternal chromosomes. This is a conflict in which maternal chromosomes are clearly the winners and paternal chromosomes the losers. As one would expect, the elimination of paternal chromosomes is controlled by the maternal genome (Haig, 1993b,c). Parental imprinting has also been predicted to occur in the workers of social insects (Haig, 1992a).

IGF-II and the IGF type 2 receptor

The reciprocal imprinting of *Igf2* and *Igf2r* in the mouse provides strong support for the genetic-conflict hypothesis, because the hypothesis predicts that imprinting will primarily affect genes that influence the cost of an offspring to its mother and that the effects of paternally expressed genes at these loci will increase the cost of an offspring whereas the effects of maternally expressed genes will decrease the cost.

Mice that inherit a disrupted paternal copy of *Igf2* are born small, and remain small into adult life. By contrast, mice that inherit a disrupted maternal copy are normal-sized. The difference in phenotype is explained by the observation that the maternal allele is not transcribed in most tissues that express IGF-II (DeChiara *et al.*, 1990, 1991). The genetic-conflict hypothesis proposes that inactivation of the maternal copy of *Igf2* was initially favored because this enabled mothers to produce larger numbers of offspring over the course of their reproductive lives. Subsequent selection would then have favored higher levels of expression by paternal alleles.

IGF-II binds to two receptors in mammals. The type 1 receptor is responsible for most of the growth-promoting effects of IGF-II. The type 2 receptor, encoded by *Igf2r*, has binding sites for mannose 6-phosphate residues in addition to its binding site for IGF-II (Humbel, 1990). The ancestral role of this molecule appears to be that of a mannose 6-phosphate receptor because the IGF-II binding site is absent from the homologous molecule in chickens and toads (Clairmont & Czech, 1989). The principal function of mannose 6-phosphate receptors is to transport molecules into lysosomes (Kornfeld, 1992).

Igf2r is exclusively expressed from the maternal allele in mice (Barlow *et al.*, 1991). Haig & Graham (1991) proposed that the maternally produced type 2 receptor functions as a 'sink' to internalize and degrade paternally produced IGF-II in lysosomes before the growth factor can bind to its type 1 receptor. At the time of this proposal, some experimental evidence suggested that the type 2 receptor had a role in the degradation of IGF-II (e.g. Oka *et al.*, 1985; Sessions *et al.*, 1987; Nolan *et al.*, 1990). This evidence has been strengthened by subsequent studies (Filson *et al.*, 1993).

Imprinting of IGF-II probably preceded the acquisition of an IGF-binding site by the mannose 6-phosphate receptor because unimprinted genes at different loci expressed in an embryo do not 'disagree' about embryonic growth rates, whereas genes at imprinted loci may disagree with genes at unimprinted loci. In this scenario, the IGF-binding site was favored because it reduced the growth-promoting effects of paternally produced IGF-II. Natural selection would then favor alleles at the receptor locus that were inactive when paternally derived. The mannose 6-phosphate receptor of opossums binds IGF-II (Dahms *et al.*, 1993). If our reasoning is correct, this implies that imprinting of IGF-II evolved before the divergence of marsupials from eutherian mammals.

Future tests

Igf2 and *Igf2r* are probably the best understood imprinted genes. We do not yet know whether the effects of other imprinted loci will conform to the predictions

of the genetic-conflict hypothesis. Cattanach *et al.* (1992) identified proximal chromosome 7 and distal chromosome 17 of mice as imprinted regions that provided apparent counterexamples to the hypothesis because a paternal duplication of these regions, with an associated maternal deficiency, is associated with poor postnatal growth. The effects of a paternal chromosome in the absence of a maternal homolog may not accurately reflect the effects of the paternal chromosome in a normal mouse. However, this argument cuts both ways and applies with equal force to imprinted regions that apparently conform to predictions. Final judgment must await further information (such as is available for *Igf2* and *Igf2r*) about the effects of the imprinted genes in these regions.

Other hypotheses

Prevention of parthenogenesis

Mouse embryos without a paternal genome do not complete development. Parental imprinting thus eliminates the possibility of parthenogenetic reproduction (Solter, 1988). If this effect is to be a function rather than a side-effect of imprinting, one must argue that the genes responsible for imprinting have been preferentially replicated *because* they prevent parthenogenesis. It is difficult to see how this could be true. A maternally imprinted gene in a parthenogenetic embryo causes the death of the embryo and thus eliminates itself. Imprinting seemingly could not evolve in the face of a gene causing females to reproduce entirely asexually.

Proponents of this hypothesis must therefore argue that the death of parthenogenetic embryos enhances the fitness of surviving sexual embryos with the imprinted allele. If a female is partly asexual, so presumably are her parthenogenetically produced daughters (and at least some of her sexually produced daughters). Parthenogenesis in one generation then does not preclude sexual reproduction in subsequent generations, and vice versa. The advantages that flow from the death of parthenogenetic embryos become harder and harder to find. These difficulties appear insurmountable when one asks how an imprinted allele could first become established in a population of unimprinted alleles. The imprinted allele would initially be present in heterozygous mothers, and whatever benefits accrued from the death of its parthenogenetic carriers would presumably be shared with the 50% of embryos that inherited the unimprinted allele from their mother. The benefits would need to be implausibly large for imprinting to be favored by natural selection.

Other considerations also argue against parental imprinting being an adaptation to ensure sexual reproduction. All that is needed to prevent parthenogenesis

is a single locus at which expression of a paternal allele is essential. The mouse genome contains several imprinted regions, all except one of which would be redundant for this function. Furthermore, the proposed function fails to explain the imprinting of genes, such as *Igf2r*, that are required maternally but not paternally. Finally, imprinting occurs in the endosperm of flowering plants but has not prevented parthenogenetic development because the embryo and endosperm are products of separate fertilizations (Haig & Westoby, 1991).

Placentation

The placenta is the structure through which an embryo obtains nutrients from its mother. The genetic-conflict hypothesis therefore predicts that imprinting will play an important role in placental development. For species with invasive placentation, maternally expressed genes are predicted to restrain – and paternally expressed genes to enhance – the invasive potential of trophoblast (Haig, 1993a). Hall (1990) and Varmuza & Mann (1994) have proposed that an important function of imprinting is to restrain the growth of placental cells. Either hypothesis would be preferable to the genetic-conflict hypothesis if it could explain the same data more simply.

Hall (1990) suggested that imprinting might be a consequence of the evolution of placentation because mammalian mothers have to tolerate the implantation of a foreign conceptus while restraining its growth. She proposed that these conflicting requirements might favor the differential functioning of the maternal and paternal genomes of the embryo and placenta. Presumably, the problem of immunological tolerance could be solved by suppressing the expression of paternal antigens (but see Kanbour-Shakir *et al.*, 1993). However, unlike the genetic-conflict hypothesis, Hall's suggestion does not specify the reasons why placental growth should be subject to imprinting, nor the direction in which placental growth factors should be imprinted.

Varmuza & Mann (1994) proposed that 'imprinting is a device that protects female mammals from the potential ravages of ovarian trophoblast disease'. In this hypothesis, the genes necessary for the development of trophoblast are inactivated in oocytes because this prevents ovarian tumors from producing invasive trophoblast. The capacity to produce trophoblast is retained by the male germline rather than the female germline because germ-cell tumors are much less frequent in males. The death of females from malignant trophoblastic disease is thus seen as the principal selective force in the origin and maintenance of imprinting. The hypothesis does not explain why genes, such as *Igf2r*, should be inactivated paternally. Varmuza & Mann suggest that paternally inactive genes may be 'innocent bystanders' caught up in the imprinting process.

This hypothesis applies at most to those mammals with invasive placentation (including mice and humans), leaving parallel phenonema in plants unexplained. Even within mammals it posits that several paternally and maternally imprinted genes have evolved in order to prevent something that requires only a single, maternally inactive gene operating in the trophoblast alone. Many species of mammals have non-invasive placentas (Mossman, 1987); we expect that imprinting will be found in these mammals as well as mice and humans (*contra* Varmuza & Mann).

Dominance modification

Sapienza (1989) proposed that imprinting was a consequence of a process of dominance modification. He believed that imprinting, by itself, would be selected against because functional hemizygosity at imprinted loci exposes loss-of-function mutants to selection. Therefore, the reason why imprinting alleles 'are maintained and appear to be prevalent in some populations must be due to functions which are independent of the effects they exert on other loci'. In other words, Sapienza believed imprinting to be a *deleterious side-effect* of some more important function of the imprinting locus. He was unclear about what this essential function might be, but suggested it could have something to do with the process of sexual reproduction, such that mutants in which the imprinting gene had been rendered non-functional would be sterile.

In Sapienza's hypothesis, imprinting is an effect of the imprinting locus (by definition). Is imprinting also an effect of the imprinted locus? As we have argued above, the answer is *Yes* if there are sequence elements of the imprinted locus that make it susceptible to the action of the imprinting locus. In this case, his hypothesis does not explain why unimprinted alleles have not arisen by mutation and spread to fixation. The answer is *No* if the imprinted locus would remain imprinted regardless of what changes were made to its sequence. In this case, his hypothesis denies the occurrence of mutations at the imprinting locus that would uncouple its essential function from its unwanted side-effects.

Host defense

Barlow (1993) suggested that 'imprinting may have evolved in mammalian oocytes as an extension of the host defense role of DNA methylation'. She proposed that the original function of imprinting loci (methyltransferases) was to inactivate foreign DNA. In her model, some host genes became subject to modification by methyltransferases because these genes had acquired sequence elements ('imprinting boxes') that resembled foreign DNA. Parent-specific

expression of the newly imprinted genes was a consequence of methylation occurring predominantly in the maternal germline.

Barlow's hypothesis addresses the mechanisms of imprinting and the evolutionary history of these mechanisms, but does not address whether imprinting is a function or a side-effect of the imprinted genes. That is, her model is not concerned with the selective (or non-selective) processes by which imprinted alleles became established and are maintained in a population. Whereas the genetic-conflict hypothesis makes claims about the function of imprinting but is silent about the mechanisms, the host-defense hypothesis is silent about functions but makes claims about mechanisms. The two hypotheses address different questions and the truth of one would not negate the other.

Gene regulation

A number of authors have proposed that the function of imprinting is to regulate gene expression and embryonic development. These proposals explain nothing unless reasons are given why some genes are imprinted but not others, why most organisms regulate development without imprinting, why imprinted genes are not regulated by some other mechanism, and so on. As Varmuza & Mann (1994) have noted, 'much more sensitive and sophisticated regulatory mechanisms have evolved that can adjust levels of gene expression by orders of magnitude, and other vertebrates seem to manage quite nicely without imprinting'.

Overview

Imprinted loci have major effects on early growth. Moreover, imprinted alleles inherited from one sex are transcriptionally inactive and are therefore exposed to negative selection when the transcriptionally active allele is a deleterious mutation. Such fitness effects argue strongly against the idea that imprinting does not require a selective explanation because genes could become imprinted as a mere side-effect of some other process. A clear distinction should be made here between the reasons for the persistence of imprinted genes and the reasons for the persistence of imprinting genes. If imprinting genes have regulatory effects on large parts of the genome, their effects on imprinted genes may indeed be a side-effect of more important functions. However, a functional explanation is still required for why genes at imprinted loci remain susceptible to imprinting. In a sense, imprinted alleles could be considered to exploit imprinting loci for their own purposes.

The genetic-conflict hypothesis proposes that imprinting evolves because of conflicts of interest between the maternal and paternal genes of an individual.

One such conflict arises when an individual's actions have opposite fitness implications for itself and for a maternal half-sib. The individual's maternal genes have a 50% chance of being present in the half-sib. Therefore, maternal genes will be selected to forgo a benefit for their own individual if this benefit is associated with a cost that is more than twice as great for the half-sib. On the other hand, the individual's paternal genes are absent from the half-sib, and will be selected to take the benefit no matter what the cost to the half-sib. During mammalian pregnancy, this conflict is mediated through demands on the mother because resources committed to one offspring become unavailable to the offspring's half-sibs. The potential for conflict is muted, but still present, if the mother's offspring include a proportion of full-sibs.

The hypothesis is compatible with multiple loci being imprinted and with some imprinted genes being maternally inactive and others paternally inactive. The expression of maternally inactive genes is predicted to increase the demands on a mother, whereas the expression of paternally inactive genes is predicted to reduce these demands. By contrast, hypotheses that imprinting has evolved to prevent parthenogenesis or to prevent the development of trophoblast in ovarian carcinomas require no more than a single maternally inactive gene. The argument that parental imprinting has evolved as a mechanism of gene regulation merely redescribes the phenomena to be explained.

The genetic-conflict hypothesis does not address the mechanisms of parental imprinting and its predictions are largely independent of the nature of these mechanisms. This independence of mechanism may be considered either a strength or a weakness of the hypothesis, depending on the question of interest. Clearly, hypotheses about the function of imprinting are not in competition with hypotheses about the mechanisms. Both kinds of hypotheses are necessary for a satisfactory understanding of imprinting.

References

Barlow, D. P. (1993). Methylation and imprinting: from host defense to gene regulation? *Science* **260**, 309–10.

Barlow, D. P., Stöger, R., Herrmann, B. G., Saito, K. & Schweifer, N. (1991). The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the *Tme* locus. *Nature* **349**, 84–7.

Cattanach, B. M. (1991). Chromosome imprinting and its significance for mammalian development. In *Genome Analysis*, vol. 2: *Gene Expression and its Control*, ed. K. E. Davies & S. M. Tilghman, pp. 41–71. New York: Cold Spring Harbor Laboratory Press.

Cattanach, B. M., Barr, J. A., Evans, E. P., Burtenshaw, M., Beechey, C. V., Leff, S. E., Brannan, C. I., Copeland, N. G., Jenkins, N. A. & Jones, J. (1992). A candidate mouse model for Prader–Willi syndrome which shows an absence of *Snrpn* expression. *Nature Genet.* **2**, 270–4.

Clairmont, K. B. & Czech, M. P. (1989). Chicken and *Xenopus* mannose 6-phosphate receptors fail to bind insulin-like growth factor II. *J. biol. Chem.* **264**, 16390–2.

Dahms, N. M., Brzycki-Wessell, M. A., Ramanujam, K. S. & Seetharam, B. (1993). Characterization of the mannose 6-phosphate receptors (MPRs) from opossum liver: opossum cation-independent MPR binds insulin-like growth factor-II. *Endocrinology* **133**, 440–6.

DeChiara, T. M., Efstratiadis, A. & Robertson, E. J. (1990). A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature* **345**, 78–80.

DeChiara, T. M., Robertson, E. J. & Efstratiadis, A. (1991). Parental imprinting of the mouse insulin-like growth factor II gene. *Cell* **64**, 849–59.

Filson, A. J., Louvi, A., Efstratiadis, A. & Robertson, E. J. (1993). Rescue of the T-associated maternal effect in mice carrying null mutations in *Igf-2* and *Igf2r*, two reciprocally imprinted genes. *Development* **118**, 731–6.

Haack, H. & Hodgkin, J. (1991). Tests for parental imprinting in the nematode *Caenorhabditis elegans*. *Molec. gen. Genet.* **228**, 482–5.

Haig, D. (1992a). Intragenomic conflict and the evolution of eusociality. *J. theor. Biol.* **156**, 401–3.

Haig, D. (1992b). Genomic imprinting and the theory of parent–offspring conflict. *Semin. devel. Biol.* **3**, 153–60.

Haig, D. (1993a). Genetic conflicts in human pregnancy. *Q. Rev. Biol.* **68**, 495–532.

Haig, D. (1993b). The evolution of unusual chromosomal systems in sciarid flies: intragenomic conflict and the sex ratio. *J. evol. Biol.* **6**, 249–61.

Haig, D. (1993c). The evolution of unusual chromosomal systems in coccoids: extraordinary sex ratios revisited. *J. evol. Biol.* **6**, 69–77.

Haig, D. & Graham, C. (1991). Genomic imprinting and the strange case of the insulin-like growth factor-II receptor. *Cell* **64**, 1045–6.

Haig, D. & Westoby, M. (1989). Parent-specific gene expression and the triploid endosperm. *Am. Nat.* **134**, 147–55.

Haig, D. & Westoby, M. (1991). Genomic imprinting in endosperm: its effects on seed development in crosses between species and between different ploidies of the same species, and its implications for the evolution of apomixis. *Phil. Trans. R. Soc. Lond.* B **333**, 1–13.

Hall, J. G. (1990). Genomic imprinting: review and relevance to human diseases. *Am. J. hum. Genet.* **46**, 857–73.

Hamilton, W. D. (1964). The genetical evolution of social behaviour. *J. theor. Biol.* **7**, 1–52.

Humbel, R. E. (1990). Insulin-like growth factors I and II. *Eur. J. Biochem.* **190**, 445–62.

Kanbour-Shakir, A., Kunz, H. W. & Gill, T. J. (1993). Differential genomic imprinting of major histocompatibility complex class I antigens in the placenta of the rat. *Biol. Reproduc.* **48**, 977–86.

Kornfeld, S. (1992). Structure and function of the mannose 6-phosphate/insulinlike growth factor II receptors. *A. Rev. Biochem.* **61**, 307–30.

Lindsley, D. L. & Grell, E. H. (1969). Spermiogenesis without chromosomes in *Drosophila melanogaster*. *Genetics* (Suppl.) **61**, 69–78.

Moore, T. & Haig, D. (1991). Genomic imprinting in mammalian development: a parental tug-of-war. *Trends Genet.* **7**, 45–9.

Mossman, H. W. (1987). *Vertebrate Fetal Membranes*. New Brunswick: Rutgers University Press.

Nolan, C. M., Kyle, J. W., Watanabe, H. & Sly, W. S. (1990). Binding of insulin-like growth factor II (IGF-II) by human cation-independent mannose 6-phosphate receptor/IGF-II receptor expressed in receptor-deficient mouse L cells. *Cell Regulation* **1**, 197–213.

Oka, Y., Rozek, L. M. & Czech, M. P. (1985). Direct demonstration of rapid insulin-like growth factor II receptor internalization and recycling in rat adipocytes. *J. Biol. Chem.* **260**, 9435–42.

Sapienza, C. (1989). Genome imprinting and dominance modification. *Ann. N.Y. Acad. Sci.* **564**, 24–38.

Sessions, C. M., Emler, C. A. & Schalch, D. S. (1987). Interaction of insulin-like growth factor II with rat chondrocytes: receptor binding, internalization, and degradation. *Endocrinology* **120**, 2108–16.

Solter, D. (1988). Differential imprinting and expression of maternal and paternal genomes. *A. Rev. Genet.* **22**, 127–46.

Varmuza, S. & Mann, M. (1994). Genomic imprinting – defusing the ovarian time bomb. *Trends Genet.* **10**, 118–23.

Appendix: functions and side-effects

A DNA sequence may have phenotypic effects, which influence the probability that the sequence itself will be replicated. Sequences that promote their own replication will be perpetuated, whereas sequences that are less effective replicators will be eliminated. The *effects* of a sequence may thus be included among the *causal* factors that account for the presence of the sequence in a gene pool. It is this causal feedback between genotype and phenotype – when combined with a source of genetic novelty (mutation) – that explains how a purposeless process (natural selection) can produce purposeful structures and functions (adaptation). The functions of a sequence consist of those of its effects that have contributed, however indirectly, to the sequence's own transmission from past generations. In so far as the future repeats the past, such functions will contribute to the sequence's transmission to future generations.

All effects of a sequence make up its phenotype, but only those effects that promote the sequence's replication make up its function. Thus, the phenotypic effects of a sequence can be classified as either *functions* (effects that are beneficial for the sequence) or *side-effects* (effects that are neutral or harmful for the sequence). For an effect to qualify as a function, variant sequences must have been eliminated in the past because they lacked the function. If an effect is to remain a function, such variant sequences must continue to be eliminated whenever they arise. Thus, a function is both a cause and an effect of the sequence.

An effect of a sequence has not yet been defined. An *effect* is simply a difference from what would be observed in the absence of the sequence or in the presence of a variant sequence, other things being equal. Thus, the effects of a sequence (and likewise its functions) depend on the implicit or explicit alternatives with which the sequence is compared. These alternatives can be narrowly or broadly defined. For example, one could argue that the maternal copy of the *Igf2* locus is inactive in mice *because* this prevents overproduction of IGF-II. The implicit comparison is to an unimprinted allele that causes no reduction in paternal expression but

increases maternal expression to the same level. A broader definition of the alternatives would compare the imprinted allele to unimprinted alleles with a range of expression levels. This comparison focuses attention on the question why expression levels are regulated by imprinting rather than by some other mechanism.