WHAT GOOD IS GENOMIC IMPRINTING: THE FUNCTION OF PARENT-SPECIFIC GENE EXPRESSION

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Parent-specific gene expression (genomic imprinting) is an evolutionary puzzle because it forgoes an important advantage of diploidy — protection against the effects of deleterious recessive mutations. Three hypotheses claim to have found a countervailing selective advantage of parent-specific expression. Imprinting is proposed to have evolved because it enhances evolvability in a changing environment, protects females against the ravages of invasive trophoblast, or because natural selection acts differently on genes of maternal and paternal origin in interactions among kin. The last hypothesis has received the most extensive theoretical development and seems the best supported by the properties of known imprinted genes. However, the hypothesis is yet to provide a compelling explanation for many examples of imprinting.

EPIGENETIC
Modifications of chromatin or DNA (for example, histone deacetylation and cytosine methylation) that can be stably transmitted through many cell divisions, but can also be reset (unlike the DNA sequence).

EVOLVABILITY
The capacity of a genetic system to generate new adaptations.

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The first use of 'imprinting' to describe EPIGENETIC parent-of-origin effects was in the context of the elimination of paternal chromosomes during spermatogenesis in sciarid flies[1,2]. In this example, imprinting referred to differences in the segregation of homologues without differences in gene expression. However, in this review, we are concerned solely with parent-specific gene expression. Typically, one allele at an imprinted locus is transcriptionally silent, with all gene products produced from the other allele (monoallelic expression); however, patterns of imprinting can be more complex, with monoallelic expression limited to some cell types, or with a mixture of maternal-specific, paternal-specific and biallelic transcripts being produced from different promoters at a single locus[3].

The most widely accepted explanation for the predominance of diploidy among complex multicellular organisms is that the possession of two functional copies of each gene masks the effects of deleterious recessive mutations[4]. In this view, monoallelic expression is paradoxical because it forgoes the advantages of diploidy. Therefore, there is a need to invoke some selective advantage of imprinting to outweigh these costs. A satisfactory explanation of this advantage faces two challenges. First, to explain the diversity of imprinted genes and their phenotypic effects (TABLE 1). Second, to explain why most loci are not imprinted. The number of imprinted loci is unknown — at present, the Harwell imprinting web site lists more than 60 imprinted transcripts in mice — but it is clear that these loci form only a minority of the mammalian genome. This conclusion is supported by the ubiquity of recessive inheritance of the phenotypes of loss-of-function mutations, both in medical genetics and in knockout mice, and the failure to detect imprinted genes until the early 1990s.

This review discusses three hypotheses that attempt to identify the selective advantage of genomic imprinting: EVOLVABILITY models propose that imprinting provides a population with enhanced adaptability to changing environments by protecting a subset of the alleles in each generation from the full force of natural selection[5,6]; the ovarian-time-bomb hypothesis (OTB) proposes that imprinting evolved to protect females from the ravages of ovarian trophoblastic disease[7]; and the kinship theory proposes that imprinting has evolved because of an evolutionary conflict in individuals between maternally and paternally derived alleles[8]. We give the most attention to the third

Table 1 | **Diverse effects of imprinted genes**

Locus	Tissue	Phenotypic effect	References
Padumnal expression			
Igf2	Placenta	Growth promotion	77
Air	Placenta	*Igf2r* imprinting	78
Peg3	Brain	Maternal care	56
SDHD	Carotid body	Oxygen sensing	79
HBII-52	Brain	RNA editing	80
Madumnal expression			
Igf2r	Placenta	Growth inhibition	81
Mash2	Placenta	Trophoblast differentiation	82
Gnas	Brown fat	Non-shivering thermogenesis	83
Tsix	Placenta	X inactivation	84
UBE3A	Brain	Speech	85

A sample of imprinted genes, with an example of a tissue in which the gene is imprinted and an associated phenotypic effect; these genes might also be expressed in other tissues and have other effects. *Air*, antisense *Igf2r* RNA; *Gnas*, guanine nucleotide binding protein α-stimulating subunit; *HBII-52*, human brain-specific small nucleolar RNA; *Igf2*, insulin-like growth-factor 2; *Igf2r*, insulin-like growth-factor 2 receptor; *Mash2*, achaete-scute complex homologue-like 2; *Peg3*, paternally expressed 3; *SDHD*, succinate dehydrogenase complex subunit-D; *Tsix*, antisense to X (inactive)-specific transcript; *UBE3A*, ubiquitin protein ligase E3A.

hypothesis because this is the theory that has been most extensively developed and, we believe, has most successfully explained the empirical phenomena.

Several other hypotheses have been advanced to explain the adaptive function of imprinting, but space does not allow us to review all these theories, particularly as many are now of only historical interest. Earlier reviews[9,10] should be consulted for a more comprehensive discussion of theories up until the mid-1990s. Of particular interest among the more recent hypotheses, is the proposal that the imprinting of X-linked loci might have evolved as a mechanism of sex-specific expression[11]. That is, both sexes possess maternally derived alleles at X-linked loci, but only females possess paternally derived alleles; therefore, imprinted expression at X-linked loci can result in differences of expression between the sexes. Here, we focus on theoretical work as it applies to autosomal loci, but the reader should keep in mind that the story might be different, and perhaps more complex, for the X chromosome.

The scope of this review is limited to questions of the FUNCTION of parent-specific gene expression, and there are many evolutionary questions about imprinting-related phenomena that we do not address. First and foremost, our review does not address the mechanisms of imprinting. Natural selection chooses among the phenotypic effects of DNA sequences. If different mechanisms have the same effects, they are subject to the same selective forces. In this sense, explanations of function are independent of questions about mechanism[9]. In particular, we use 'silencing' to refer to the evolutionary process by which a locus that was initially expressed from both alleles comes to be expressed from a single allele, with the other allele silent. This process is predicted to involve the increased expression of one allele and the decreased expression of the other (BOX 1). Our usage of 'silenced' is not intended to imply anything about the physical mechanism by which monoallelic expression is achieved in each generation (that is, whether transcriptional activity or transcriptional silence is the default state).

Recent theoretical papers that address issues that are not covered by this review include hypotheses about the function of parent-specific epigenetic differences that are not associated with differences in gene expression[12], the causes of random monoallelic expression and how these provide a pool of genetic variability from which parent-specific monoallelic expression could evolve[13], and the kinds of mutation that give rise to imprinted expression[14]. Other reviews are recommended for an introduction to the mechanisms of imprinting[15] and the physiological functions of imprinted genes[16].

Enhanced evolvability

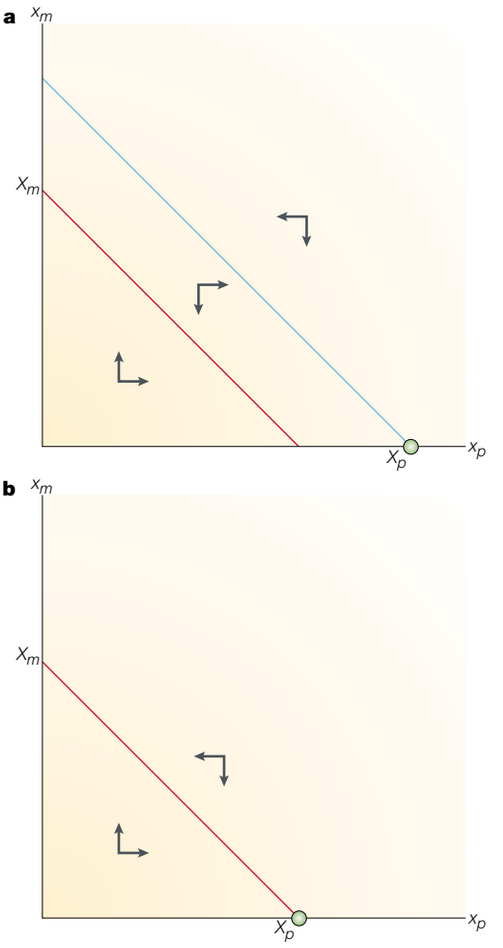
McGowan and Martin[5], and Beaudet and Jiang[6] have proposed that imprinting has evolved because functional haploidy confers increased evolvability on a population. In each generation, one of the two alleles at a locus is masked from the scrutiny of natural selection. Furthermore, a subset of alleles will, by chance, spend several consecutive generations in the silent state. During this period, the masked alleles can accumulate many mutations. This is proposed to increase the rate of adaptive evolution because it increases the probability of adaptive changes that require synergism between two or more individually deleterious mutations and/or because it shields temporarily deleterious alleles from selective elimination in fluctuating environments. So, genomic imprinting is seen as contributing to the accumulation of a pool of hidden variability that provides a selective advantage to the group in the face of a changing environment.

It is unclear whether such models can work. The effects of most single mutations — and most double, triple or quadruple mutations — are deleterious. Moreover, for most silent alleles at an imprinted locus, the number of generations since the allele was last active is small, and the chance of multiple mutations having occurred during this period is also small. In fact, for a recessive mutation, genomic imprinting reduces the expected number of generations before the mutation is exposed to selection. Put another way, the equilibrium frequency of deleterious recessives is higher for biallelic than for monoallelic expression[17]. Beaudet and Jiang[6] explicitly state that the benefit of enhanced adaptability accrues to the group rather than to individuals, but their model has little to say about how imprinting first becomes established in groups and how the group benefit of imprinted expression is maintained in the face of individual benefits from reversion to biallelic expression. Perhaps these difficulties can be surmounted, but it is not obvious how, and it would require a more formal quantitative analysis than any yet presented.

Even if evolvability models can be made to work, the models will face the problem of explaining which loci would be imprinted, and why most loci are not imprinted. Beaudet and Jiang[6] propose that imprinting

FUNCTION
The phenotypic effects of a DNA sequence that are responsible for the selective maintenance of its integrity in the face of mutational processes.

Box 1 | **Evolutionary equilibria at an imprinted locus**



Many criticisms of the kinship theory result from simple misunderstandings of the nature of the evolutionary equilibrium that it predicts. Suppose that fitness is determined by the level of production of a growth factor, and that natural selection favours higher production when an allele is paternally derived than when it is maternally derived. Overall production (X) is the sum of production by the madumnal (maternally derived) allele (x_m) and the padumnal (paternally derived) allele (x_p). In panel **a**, the x_m,x_y-plane is dissected by two lines. On the red line, x_m and x_p sum to the madumnal optimum X_m. On the blue line, x_m and x_p sum to the padumnal optimum X_p. To the right of the blue line, selection favours mutations that reduce both x_m and x_p (indicated by black arrows). To the left of the red line, selection favours mutations that increase both x_m and x_p. Between the lines, selection favours mutations that reduce x_m but increase x_p. A population is in evolutionary equilibrium when $x_p = X_p$ and $x_m = 0$ (green spot) because at this point, both increases and decreases in x_p would be selected against, as would increases in x_m. The approach to this equilibrium from an unimprinted state probably involves increases in x_p and decreases in x_m.

If natural selection favours the higher production of a growth inhibitor when alleles are maternally derived ($X_m > X_p$), then the red line would be shifted to the right of the blue line. The evolutionary equilibrium in this case would be $x_p = 0$ and $x_m = X_m$. So, whichever allele favours higher production is predicted to produce its favoured amount, with the other allele silent. This prediction assumes that the two alleles contribute their product to a common pool, the size of which determines fitness. The assumption is violated at loci that are subject to random X inactivation because the two alleles are expressed in different cells[29].

At the evolutionary equilibrium of panel **a**, reactivation of the silent madumnal allele (or paternal UNIPARENTAL DISOMY) would result in total production $2X_p$, whereas inactivation of the active padumnal allele (or maternal uniparental disomy) would result in zero production. Both kinds of 'mutation' shift production outside the zone of conflict. Therefore, care should be used in interpreting the resulting phenotypes in terms of the kinship theory.

Suppose that a population with a promiscuous mating system changed to strict monogamy. Madumnal and padumnal alleles would now both favour X_m. In the new selective environment (panel **b**), all points on the red line are possible equilibria. Over the long term, populations might be expected to drift along this line, with a weak bias towards the equal expression of the two alleles. However, in the initial transition from the selective environment of panel **a** to that of panel **b**, natural selection would favour decreases in x_p without reactivation of the silent madumnal allele. Despite contrary claims[53], the kinship theory does not predict a rapid loss of imprinting.

UNIPARENTAL DISOMIES
Both copies of a chromosome derived from one parent.

TERATOMA
A tumour consisting of several cell types.

TROPHOBLAST
The extraembryonic cell population at the maternal–fetal interface. In mice and humans, elements of the trophoblast invade the maternal tissues of the uterus.

should evolve at dosage-sensitive loci "that generate a phenotypic continuum, without unrelated deleterious effects", and that this category might be expected to include genes that affect growth and certain behaviours — such as level of activity — but these criteria are vague. The patchy phylogenetic distribution of imprinting is also problematic for these theories, because the proposed selective advantage should be equally valid for any diploid organism. Finally, the models themselves provide no methods by which to predict in which germline — male or female — an allele should be silenced.

The ovarian time bomb

Ovarian teratomas arise when an unfertilized oocyte spontaneously initiates development. TERATOMAS produce most tissue types, but are relatively benign because they fail to differentiate into invasive TROPHOBLASTS . This failure is plausibly explained by a requirement of paternally derived genes for normal development of the trophoblast. Varmuza and Mann[7] proposed that this consequence of imprinting was also its function. In their view, the genes that are responsible for trophoblast development are inactivated in the oocytes to prevent ovarian trophoblastic

Box 2 | **Parent-specific inclusive fitness**

In an often-repeated anecdote, the British geneticist J. B. S. Haldane expressed a willingness to give his own life to save more than two brothers or more than eight cousins. The manner in which genomic imprinting modifies calculations of inclusive fitness can be illustrated by asking the question, would Haldane have given his life to save three half-brothers? For this purpose, assume that the four half-brothers (including Haldane) have the same mother but different fathers, and have identical reproductive prospects. A madumnal (maternally derived) allele in Haldane has one chance in two of being present in each half-brother. Therefore, for the sacrifice of one copy of itself in Haldane, the allele could expect to save one and a half copies in the three half-brothers. From a genetic perspective, the sacrificial act is a good deal. However, a padumnal (paternally derived) allele in Haldane is necessarily absent from the half-brothers, and there is no recompense for the sacrifice of its single copy in Haldane. Seemingly, there is an internal conflict in Haldane over the desirability of the sacrificial act. Formally, the cost of the sacrifice of Haldane ($C = 1$) is weighed against the benefit of the lives of the three half-brothers ($B = 3$). The coefficient of matrilineal relatedness (r_m) of a maternal half-brother is a half, but the coefficient of patrilineal relatedness (r_p) is zero. Therefore, the matrilineal inclusive fitness effect of the self sacrifice is positive ($r_mB - C = 0.5$), but the patrilineal inclusive fitness effect is negative ($r_pB - C = -1$). In the absence of genomic imprinting, the parental origin of an allele is unspecified and the appropriate coefficient of relatedness is the average of r_m and r_p (that is, $r = 1/4$). In this case, the inclusive fitness effect is negative ($rB - C = -0.25$). An unimprinted gene that was expressed in a long series of 'Haldanes' would be maternally and paternally derived with equal frequency and, on average, would not benefit from repeated sacrificial acts.

disease. The active copies of these genes, which are necessary for successful implantation, are then provided by the sperm genome after fertilization. A weakness of this hypothesis was that it did not explain the imprinting of genes that were not involved in trophoblast development, neither did it explain the inactivation of some genes in paternal germlines. In anticipation of this criticism, Varmuza and Mann[7] suggested that many genes might be 'innocent bystanders' that become imprinted when they are inadvertently recognized by the imprinting machinery.

The OTB has now been formally modelled. These models show that the hypothesis can explain the silencing of maternally derived alleles at loci that encode enhancers of trophoblast growth[18–20] and can also explain the silencing of paternally derived alleles at loci that encode suppressors of trophoblast growth, without a need to invoke their status as innocent bystanders[18,20]. The OTB has, therefore, been shown to entail no logical contradictions, if the assumptions of the hypothesis are met. The OTB can also predict the directionality that is observed in the growth-related effects of maternally silenced and paternally silenced genes in mammals with an invasive trophoblast.

The OTB has limitations, however, as a general theory of the evolution of imprinting. First, the hypothesis invokes bystander effects to explain the imprinting of genes that do not have a role in trophoblast development. Second, the prediction of the OTB that growth enhancers should be silenced in female germlines, but active in male germlines, is contingent on the observation that germcell tumours are more common in females than in males[7]. However, the greater malignancy of male germ-cell tumours might be the cause of their lower frequency, rather than the reverse. That is, if the inactivation of growth factors in female germlines arose for some other reason, germ-cell tumours would be more benign in females than in males. The higher metastatic potential of testicular germ-cell tumours would then result in stronger selection to reduce their frequency, whereas selection to prevent the comparatively benign ovarian tumours would be relaxed. Third, imprinting occurs in taxa that lack invasive placentas. For example, imprinting of insulin-like growth-factor 2 (*IGF2*) and of insulin-like growth-factor 2 receptor (*IGF2R*) has been reported in marsupials[21,22], but invasiveness is not a prominent feature of the CHORIOVITELLINE placentas of most marsupials[23]. Imprinting has also been observed in eutherian mammals with non-invasive placentas, such as sheep[24]. Fourth, the persistence of monoallelic expression in somatic tissues, and the loss of the advantages of diploidy, must be viewed as non-adaptive by-products of selection to prevent ovarian trophoblastic disease. By contrast, the kinship theory (discussed below) — which also predicts the opposite imprinting of enhancers and inhibitors of trophoblastic growth — can explain the imprinting of genes that do not affect the trophoblast without an appeal to bystander effects.

The kinship theory of genomic imprinting

The kinship theory of genomic imprinting is more commonly known as the conflict theory. However, we refer to it as the kinship theory because it is the appeal to effects on KIN that is the distinctive feature of the hypothesis. Our review of this theory starts with a brief discussion of the concept of inclusive fitness, and how genomic imprinting necessitates a reappraisal of this concept. We then discuss the theoretical elaborations of the kinship theory that have been made since its initial formulation, followed by a discussion of the empirical and theoretical criticisms of the theory. Haig[25] introduced the terms 'madumnal' and 'padumnal' to refer to maternally and paternally derived alleles that are present in offspring, as distinct from 'maternal' alleles that are present in mothers, and 'paternal' alleles that are present in fathers. We follow this convention in the remainder of the review.

CHORIOVITELLINE
A placenta that is derived from the fusion of the extraembryonic yolk sac and the chorion.

KIN
Individuals that share some of their genes by recent common descent.

Kinship and genomic imprinting. Genes in the liver do not leave direct genetic descendants. Nevertheless, complex patterns of liver-specific expression have evolved that favour the transmission of identical-by-descent copies of these genes through the germ cells of an individual. This same logic applies to interactions among individuals: a gene in the liver of one individual might be favoured by natural selection if it promotes the transmission of identical-by-descent copies of itself through the germ cells of another individual.

Hamilton[26] formalized this intuitive argument in his concept of inclusive fitness (BOX 2). In broad terms, the inclusive fitness effect of a gene is a sum of the effects of the expression of the gene in one individual on the fitness of all other individuals, in which the contribution of each individual to the sum is weighted by the probability (r) that the individual possesses an identical-by-descent copy of the gene. So, effects on the individual in which the gene is expressed are given full weight ($r = 1$), effects on non-relatives are given zero weight ($r = 0$) and effects on relatives are given intermediate weight, in proportion to their proximity of relationship to the first individual (r is known as the coefficient of relatedness). For example, effects on the fitness of a brother or sister ($r = 0.5$) are given half the weight of effects on the personal fitness of the individual. Hamilton showed that a gene will increase in frequency if its inclusive fitness effect is positive, but will decrease in frequency if the effect is negative.

The kinship theory recognizes that the coefficient of relatedness of individuals might differ for the madumnal and padumnal alleles at a locus[27,28]. For example, the traditional coefficient of relatedness for a maternal half-sibling was $r = 1/4$, but this coefficient can be viewed as an average of two parent-specific coefficients: a coefficient of matrilineal relatedness $r_m = 1/2$, and a coefficient of patrilineal relatedness $r_p = 0$ (BOX 2). Madumnal-specific expression is favoured if the expression of an unimprinted allele would have a positive inclusive fitness effect when maternally derived (calculated using coefficients of matrilineal relatedness) but a negative inclusive fitness effect when paternally derived (calculated using coefficients of patrilineal relatedness). Padumnal-specific expression is favoured when these relationships are reversed[27,29].

The kinship theory was initially formulated in the context of genes that are expressed in fetal tissues that affect the resources acquired from a mother. In the simplest formulation, extra resources are of direct benefit (B) to the fetus but involve an indirect cost (C) to other offspring from the same mother. These other offspring will be more likely to carry copies of the madumnal allele of the fetus than its padumnal allele ($r_m > r_p$) because of the possibility of multiple paternity of the offspring. Therefore, madumnal and padumnal alleles are in conflict over whether to take the resource from the mother when $B - r_m C < 0 < B - r_p C$. A different way to frame the problem[30,31] is to view the extra resources as of direct benefit (B) to the fetus but with costs to the residual fitness of its mother (C') and father (C''). In general, $C' > C''$, because not all of the costs to the residual fitness of the mother are associated with a cost to the residual fitness of the father. Therefore, madumnal and padumnal alleles of the fetus are in conflict over whether to take the resource whenever $B - C' < 0 < B - C''$. In this method of accounting, the cost to the mother is given full weight ($r_m = 1$) and the cost to the father is given zero weight ($r_m = 0$) when calculating the matrilineal inclusive fitness effect, but these weightings are reversed when calculating the patrilineal inclusive fitness effect.

Both methods of accounting lead to predictions that imprinted growth enhancers will be madumnally silent, whereas imprinted growth inhibitors will be padumnally silent. These predictions are based on three assumptions. First, females sometimes have offspring by more than one male. Second, the costs of the growth of an offspring fall preferentially on its mother, rather than its father. Third, genes that are expressed in offspring can influence the distribution of maternal resources. This third assumption is violated in taxa that lack postzygotic maternal care; therefore, imprinted effects on growth are not expected in such taxa.

In its most general form, the kinship theory is not just about growth, the relations between mothers and offspring, or competition among the offspring of one mother — it is a theory that explains the direction of natural selection in all social interactions among individuals that have different probabilities of sharing their madumnal and padumnal alleles. In this general theory, the inclusive fitness effect for madumnal alleles is a summation of the effects on all of the matrilineal kin of an individual (the mother, maternal half-siblings, aunts, uncles, grandparents and so on) weighted by coefficients of matrilineal relatedness, whereas the inclusive fitness effect for padumnal alleles is a summation of the effects on all patrilineal kin weighted by coefficients of patrilineal relatedness. Some individuals, such as self, full-siblings and offspring, belong to both sets of kin. The mother/offspring case has received the most attention because this is an important relationship in the lives of all mammals and is a relationship in which differences between r_m and r_p are large. However, the theory has also been applied to asymmetries of relatedness in social groups that arise because of sex-biased dispersal[28,32], and to asymmetries in families that arise from inbreeding[33,34].

Conflict resolution. Game-theoretic[25,27,35] and quantitative-genetic models[36,37] of the evolution of genomic imprinting have consistently found that whichever allele — madumnal or padumnal — favours the larger amount of a given gene product will produce this amount at evolutionary equilibrium, and the other allele will be silent (BOX 1). This property has been called the 'loudest-voice-prevails' principle[25] and can be considered a simple form of conflict resolution in which the allele that favours the higher amount presents the other allele with a *fait accompli*.

At a single locus, the kinship theory predicts the silencing of alleles when they are inherited through sperm or eggs. The co-evolution of imprinting at two

loci with antagonistic growth effects has also been modelled[35,38,39]. These models find that the loudest voice prevails at both loci, with silencing of the madumnal allele of growth promoters and the padumnal allele of growth inhibitors. There is, however, an important asymmetry between the two types of locus. In the absence of imprinting of a growth promoter, there is no selective force that favours the production of a growth inhibitor (assuming that such a growth inhibitor is initially unimprinted)[9,39]. This asymmetry indicates an evolutionary scenario for the evolution of imprinting in the *IGF2*/*IGF2R* system (BOX 3). The initial event would have been the evolution of madumnal silencing of *IGF2*, followed by the acquisition of an IGF-II-binding site by the receptor. The origin of this binding site would then have created the selective forces that favoured padumnal silencing of *IGF2R* (REF. 39). Comparative data do not allow this sequence of events to be tested because neither locus is imprinted in monotremes, and the IGF-II-binding site is absent, whereas all the components of this system are present in marsupial and eutherian mammals[22,40,41].

Conflict between imprinted and imprinting genes. The establishment of an imprint in a parental germline will result from the interaction of *trans*-acting components of the imprinting machinery with *cis*-acting elements at the imprinted locus. Alleles at the loci that are responsible for the *trans*-acting factors will usually segregate independently of alleles at the imprinted locus. This difference in transmission can result in an evolutionary conflict between the imprinted loci (expressed in offspring) and the imprinting loci (expressed in parents)[30,42,43]. A rare variant allele at one of the *trans*-acting loci will generally affect expression in all of the offspring of a parent, whereas a rare variant at one of the *cis*-acting loci will directly affect expression only in the offspring that inherit the variant. In short, imprinted loci evolve according to madumnal and padumnal interests, whereas imprinting loci evolve according to maternal and paternal interests.

Wilkins and Haig[43] have argued that this conflict between *cis*- and *trans*-acting factors is likely to be expressed in paternal germlines, and to affect the padumnal silencing of DEMAND INHIBITORS, but not in maternal germlines in which imprinting results in the madumnal silencing of DEMAND ENHANCERS. The reason is that maternal genes favour lesser demands by offspring than either madumnal or padumnal genes. Therefore, if a demand enhancer is madumnally silent in the offspring, there is no evolutionary incentive for maternal *trans*-acting factors to reactivate the silent madumnal allele. Paternal genes also favour lesser demands on mothers than do padumnal genes, if fathers have some chance of sharing some of the other offspring produced by the mother. If the proportion of shared offspring is high enough, paternal genes will also favour lesser demands than madumnal genes. Therefore, paternal *trans*-acting factors will sometimes have an evolutionary incentive to reactivate the silenced padumnal alleles of demand inhibitors.

This argument indicates that the epigenetic silencing of demand inhibitors in paternal germlines will be evolutionarily less stable than the epigenetic silencing of demand enhancers in maternal germlines. This provides a possible explanation for the observation that most madumnally silent loci are silenced by the methylation of a madumnal sense promoter, whereas most padumnally silent loci seem to be silenced indirectly by the methylation of the madumnal promoter of an antisense transcript[43,44]. The kinship theory predicts that these padumnally expressed antisense transcripts function as madumnally silent demand enhancers.

Criticisms of the kinship theory

The kinship theory has been criticized on two fronts. The first involves theoretical challenges to the structure of the theory. The second involves questions about whether observed phenomena support or contradict the predictions of the theory. We will first address the theoretical challenges before turning to an evaluation of the empirical support for the theory.

Theoretical challenges. The presentation of the kinship theory in previous sections is largely the result of game-theoretic models. However, different predictions have been extracted from models in formal population genetics[45,46]. This disparity reflects a difference in methodological approaches to the study of genetic evolution. It is a simplification to say that the approach of population genetics is to consider a small number of alleles — usually two — and to describe their change in relative frequency over time. By contrast, game-theoretic models of the kinship theory have used the criterion of non-invasibility; that is, the models find an allele, from among a large number of alternatives, that cannot be displaced by any rare alternative if the allele is near fixation in a population. These two approaches reflect different concepts of evolutionary equilibrium, with the latter tending to be associated with longer timescales during which the input of new mutations becomes important[47,48].

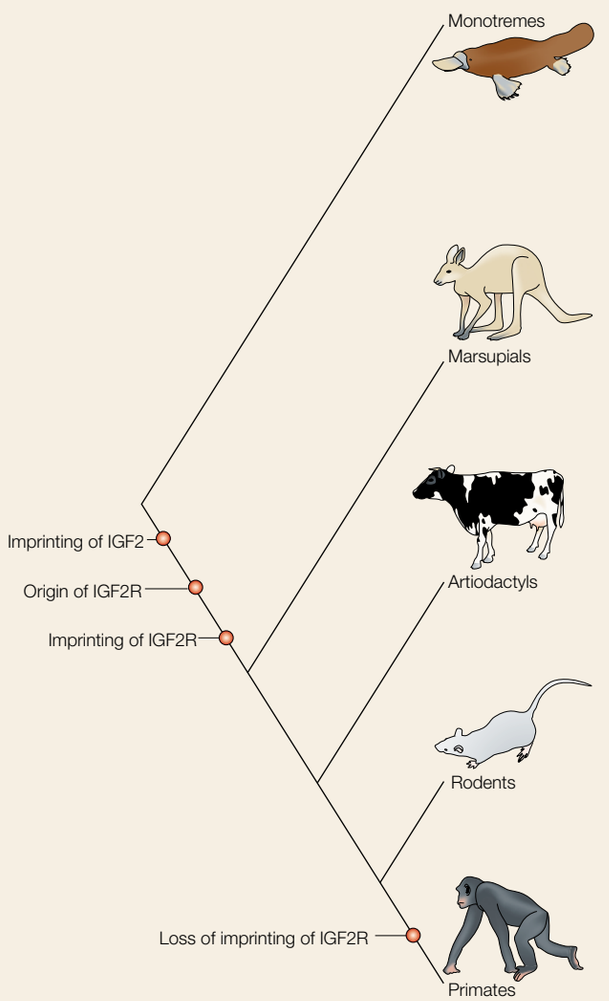
There is much common ground between the two approaches. Population-genetic models show that effects on kin do make a difference, and there are sets of parameter values for which population-genetic models give results that are consistent with the game-theoretic results. However, there are also sets of parameter values for which an imprinted allele can displace an unimprinted allele in the absence of genetic conflict, for which kinship considerations do not seem to make a difference and for which an imprinted and an unimprinted allele can coexist at a stable equilibrium[45].

We believe that the population-genetic results are misleading in two ways. First, some pairs of alleles that are considered by population-genetic models might be unlikely to compete in natural populations; for example, Spencer *et al.*[45] found that multiple paternity of the offspring of a female did not affect the dynamics of an interaction between an unimprinted

DEMAND INHIBITOR
A factor that is produced by an offspring reducing the 'demand' on its mother. That is, the production of the factor decreases the individual fitness of the offspring at a benefit to the expected fitness of its mother from other offspring.

DEMAND ENHANCER
A factor that is produced by an offspring increasing the 'demand' on its mother. That is, the production of the factor increases the individual fitness of the offspring at a cost to the expected fitness of its mother from other offspring.

Box 3 | **Imprinting of *IGF2* and *IGF2r***



Insulin-like growth-factor 2 (*Igf2*) and insulin-like growth-factor 2 receptor (*Igf2r*) were the first imprinted loci to be identified in mice. *Igf2* is expressed only from the padumnal (paternally derived) allele[65], whereas *Igf2r* is expressed only from the madumnal (maternally derived) allele[66]. Inactivation of the padumnal allele of *Igf2* results in mice that are 60% of the normal birthweight, whereas inactivation of the madumnal allele of *Igf2r* results in mice that are 140% of the normal birthweight[67]. So, in terms of their primary phenotypic effects, *Igf2* and *Igf2r* fit well with the predictions of the kinship theory[68].

As their names suggest, *IGF2* and *IGF2R* are functionally related. IGF-II binds to two receptors in mammals[69]. The type 1 receptor, encoded by *IGF1R*, mediates the growth-enhancing effects of IGF-II. The type 2 receptor, encoded by *IGF2R*, binds IGF-II at the cell surface. The ligand–receptor complex is then internalized and targeted to lysosomes in which IGF-II is degraded. As well as its IGF-II binding site, the type 2 receptor has further binding sites for phosphorylated mannose residues[70], which allow it to target mannose 6-phosphate (M6P)-labelled ligands to lysosomes. This is probably the ancestral function of the receptor, because the M6P-binding site has been found in all the vertebrates that have been investigated so far, whereas the IGF-II binding site is present in marsupials and eutherian mammals[71,72], but is absent in chickens, frogs and monotremes[40,73].

IGF2 is imprinted in marsupials, rodents, artiodactyls and primates, but not in monotremes or birds, whereas *IGF2R* is imprinted in marsupials, rodents and artiodactyls, but not in monotremes, birds, primates and their closest relatives — the tree-shrews and flying lemurs[21,22,41,74]. This phylogenetic distribution can most parsimoniously be explained by the origin of imprinting at both loci, and the acquisition of an IGF-II binding site by the mannose 6-phosphate receptor, in an ancestor of marsupials and eutherians, followed by the loss of imprinting at the *IGF2R* locus in primates and their relatives (as shown in the figure), possibly as a result of conflict between *cis*- and *trans*-acting components of the imprinting machinery[39,43].

The phylogenetic data indicates that imprinting is associated with viviparity, and is absent in oviparous vertebrates. This is broadly consistent with the predictions of the kinship theory. However, it should be noted that there is extensive post-zygotic provisioning in monotremes, both in the uterus[75] and during lactation[76]. Therefore, there is the potential for the padumnal genome to influence maternal provisioning in monotremes, just as in marsupial and eutherian mammals. Moreover, the evidence for absence of imprinting in other oviparous vertebrates is largely an absence of evidence.

allele and an imprinted allele, in a model in which the effects of the allele were indistinguishable when paternally derived, but not when maternally derived. We would argue that the parameter values that favour the success of the imprinted allele in these models would probably arise only in the presence of multiple paternity. Second, the equilibria attained in the two-allele population-genetic models need not be stable to the introduction of a third allele with a new set of parameter values. Therefore, these equilibria are unlikely to be stable in the long term, and we believe that the best predictions for natural populations are provided by the criterion of non-invasibility. However, proponents of the population-genetic approach point out that game-theoretic models ignore the dynamics of gene frequency change. The fact that an allele would be stably maintained if it were the predominant allele in a population does not necessarily mean that the allele would ever get close to fixation[20].

Conflict between these two methodological approaches is widespread in evolutionary theory, and not restricted to debates about the evolution of imprinting. Both approaches have their strengths and limitations, and the selection of one approach over the other must be based on the nature of the question being asked. We believe that the forces that favour the acquisition and maintenance of imprinting are best understood as a long-term evolutionary process that occurs on a phylogenetic timescale, and that these processes have been dominated by the introduction of new alleles, rather than changes in the frequency of existing ones.

Uniparental disomies. Hurst and McVean[49] reviewed the growth effects of uniparental disomies (UPDs) in mice and humans, and found these to be generally non-supportive of the kinship theory because they interpreted the theory as predicting overgrowth in paternal UPDs but undergrowth in maternal UPDs. Contrary to this prediction, they found that most paternal UPDs

were associated with reduced growth or no phenotype. Haig[29] argued that these comparisons are misleading, because the kinship theory predicts the fitness effects of small changes in gene expression, not the effects of doubling the dose of an imprinted gene product or of totally extinguishing its production (BOX 1). Therefore, the interpretation of UPDs (and knockout mutations) should be approached with caution because these involve large perturbations in expression, not the tinkering at the margins invoked by the kinship theory.

Iwasa *et al.*[37] presented an explicit model that attempted to illustrate how paternal UPDs could be growth retarded, and yet still be consistent with the theory. In this model, expression level at a locus determined the fractional allocation of resources in an offspring to embryonic and placental growth, with final offspring size maximized by some intermediate allocation to placental growth, and with madumnal alleles favouring a lesser allocation than padumnal alleles. At evolutionary equilibrium, padumnal alleles achieved their optimal allocation, with madumnal alleles silent (an expression of the loudest-voice-prevails principle). However, paternal UPD could result in an overallocation of resources to placental development, resulting in embryonic growth retardation.

Such arguments remove some of the sting from the UPD data for proponents of the kinship theory, but they do not completely resolve the issue. UPDs might not provide strong evidence against the theory, but they definitely cannot be interpreted as providing strong evidence in its favour. This might change as more is learned about the phenotypes of UPDs and about the underlying molecular mechanisms.

Monogamous mice. The kinship theory predicts that the level of demand that is imposed on mothers by padumnal alleles should increase with the frequency of multiple paternity of the offspring. In apparent support of this prediction, F_1 hybrids between *Peromyscus maniculatus*, a mouse with a high rate of multiple paternity, and *Peromyscus polionotus*, a mouse with a low rate, are small if the mother is *P. maniculatus*, but are large in the reciprocal cross[50]. Imprinted loci make a significant contribution to these differences between reciprocal F_1 hybrids[51,52], and these growth phenotypes could be interpreted as supporting the kinship theory. However, Hurst[53,54] interpreted these crosses as problematic for the hypothesis, because imprinting was present in the 'monogamous' *P. polionotus*, even though the interests of madumnal and padumnal genomes are identical under strict lifetime monogamy.

The kinship theory has addressed this criticism in two ways. The first is to question whether *P. polionotus* is truly monogamous[55], because rates of partner change between successive litters might be as high as 20% (REF. 56). The second is to note that the kinship theory does not, in fact, predict that an evolutionary switch to strict monogamy should be rapidly followed by a loss of imprinting[57]. In the absence of conflict, madumnal and padumnal alleles 'agree' about their combined level of production, but are largely indifferent about how this production should be divided between the two alleles (BOX 1).

Hurst[53,54] counters these arguments by invoking the costs of functional haploidy, which should favour a loss of imprinting. However, the selective forces that favour biallelic expression at an imprinted locus have been shown to be weak — of the same order as the mutation rate[42]. It should be noted that this conclusion is based on the consideration of germline mutations only. The fitness costs of functional haploidy that are associated with somatic mutations might provide a stronger selective force, but this has yet to be formally modelled.

Post-weaning effects. The selective forces that favour the evolution of imprinting in the relations of an offspring with its mother are seemingly absent once the offspring reaches nutritional independence. However, many imprinted genes do not have effects on preweaning growth, or have effects that persist after weaning. Hurst and McVean[54] interpret such effects as further evidence against the kinship theory. Before discussing the specific case of the imprinting of genes with effects on maternal behaviour, we will make two general points. First, the kinship theory applies to all of the interactions among kin, not just to the interactions between mothers and offspring during growth. Therefore, post-weaning effects are not, in principal, incompatible with an adaptive explanation in terms of the kinship theory. Second, if an imprinted gene has multiple pleiotropic effects, not all of these effects need be explained by the theory. For example, the effects before and after weaning can be considered to be instances of pleiotropy. Imprinting at a locus might persist after weaning, even though imprinting evolved because of effects before weaning. If madumnal and padumnal alleles have the same optimal level of the gene product after weaning, they will be close to indifferent about how this level of production is divided between the two alleles (BOX 1). The argument from pleiotropy is weakened, but not negated, by the existence of complex patterns of tissue-specific and stage-specific imprinting at some loci.

Among the post-weaning effects that have been interpreted as contradicting the kinship theory are defects in maternal behaviour that are shown by female mice with knockouts of two padumnally expressed genes — mesoderm specific transcript (*Mest*) and paternally expressed 3 (*Peg3*) (REFS 58,59). These effects pose a challenge to the theory because the madumnal and padumnal alleles of a mother are equally likely to be transmitted to each of the ova, and so would seem to benefit equally from the level of her maternal care[60,61].

Mest and *Peg3* have effects on prenatal growth that are consistent with the kinship theory[58,59]. Therefore, the fact that the pleiotropic effects on maternal care are also subject to imprinting could be dismissed as an unselected epiphenomenon. Of greater interest, however, are hypotheses that attempt to find a hidden asymmetry in the selective forces that act on alleles of different parental origin. One route is to propose that the care lavished on offspring by their mother has indirect costs

for other relatives who have different probabilities of carrying the madumnal and padumnal alleles of the mother. For example, if care for own offspring has fitness costs for a maternal half-sister, then a padumnal allele of the mother will favour higher levels of maternal care than will a madumnal allele[55]. Another route is to find an asymmetry of relatedness to the offspring of a mother. For example, if a mother mates with her own father, her padumnal allele will be transmitted to more of her offspring (through both ova and sperm) than will her madumnal allele (only through ova). If subsequent litters are less likely to be inbred, then her padumnal alleles will favour greater investment in the present litter than will her madumnal alleles[34]. The underlying assumptions of these hypotheses must be tested before the effects on maternal care can be interpreted as supporting the kinship theory.

Conclusions

An important weakness of evolvability models is their lack of specificity. The putative advantages of monoallelic expression should apply at most loci and in most organisms. By contrast, an important weakness of the OTB is its over-specificity. The hypothesis applies only to genes that regulate trophoblast growth in mammals. Parent-specific gene expression occurs in plants as well as mammals, and affects genes that are expressed in many tissues — a pattern that is not easily explainable by the OTB; however, it is also restricted in its occurrence to a minority of loci and seems to be absent in many taxa — a pattern that is not easily reconciled with evolvability models. On these counts, the kinship theory has had some success. As with the OTB, the kinship theory is able to explain the apparent correlation of padumnal expression with growth enhancers, and of madumnal expression with growth inhibitors. But, unlike the OTB, the kinship theory can explain the imprinting of genes in non-invasive tissues, as well as the phylogenetic association of an important role of imprinting in normal development with viviparity. Such a role has been described in plants[62–64] as well as mammals, but is apparently absent in oviparous model organisms such as *Caenorhabditis*, *Drosophila* and *Danio* (zebrafish). However, the theory has been criticized for its failure to provide a ready explanation for all the examples of imprinting.

The kinship theory has both a weak and a strong version. The weak version is an extension of the inclusive fitness theory to include expression strategies that depend on the parent of origin of an allele. Specifically, the effects of an allele that is paternally derived are subject to selection solely on their consequences for patrilineal kin, whereas the effects if it is maternally derived are subject to selection solely on their consequences for matrilineal kin[27,29]. The weak version of the theory is compatible with non-kinship factors having an important role in the evolution of imprinting, but if imprinted expression evolves — for whatever reason — the logic of the theory must apply whenever the expression of an allele has consequences for asymmetric kin (that is, individuals for whom $r_m \neq r_p$). The weak version of the theory could be falsified by being shown to involve some logical flaw, but is not vulnerable to empirical falsification. We believe that its theoretical foundations are sound.

The strong version of the theory proposes that effects on asymmetric kin have been the predominant selective force favouring the evolution of imprinted expression. Whereas the success or failure of the weak version will be determined by theoretical arguments, the strong version is a hostage to increasing knowledge about the functions of imprinted genes. In this review, we have argued that many observations that have been claimed to contradict the strong version are equivocal. However, the strong version is yet to meet the challenge of explaining the evolution of imprinting at most imprinted loci. We believe that this challenge can be met. Future research into the function of imprinted genes will tell whether this belief is justified. Before rushing to make a judgement on the success or failure of the strong version of the theory, the risk of prematurely rejecting the hypothesis, if it is correct, must be weighed against the risk of prematurely accepting the hypothesis, if it is false.

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Online links box

DATABASES

The following terms in this article are linked online to:

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink
IGF1R | *Igf2* | *IGF2* | IGF-II | *Igf2r* | *IGF2R* | *IGF2R* | *Mest* | *Peg3*

OMIM: http://www.ncbi.nlm.nih.gov/Omim
ovarian teratomas | testicular germ-cell tumours

FURTHER INFORMATION

Harwell imprinting web site:
http://www.mgu.har.mrc.ac.uk/imprinting/imprin-contact.html

Access to this interactive links box is free online.