12 Evolutionary theories of genomic imprinting

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1. Introduction

As deleterious recessive somatic mutations are hidden in diploids but not in haploids, and as most mutations are probably deleterious recessives, a multicellular organism is usually better off with two sets of chromosomes rather than one (for references and discussion see ref. 1). Why then do some genes (i.e. imprinted genes) behave as though they are haploid, with the same copy inactive in many tissues?

A variety of hypotheses have been proposed to account for the evolution of imprinting. It is the function of this chapter to critically review and evaluate these. This is prefaced, however, with a brief word on how this chapter is different from the others in the book.

2. Non-competing theories of imprinting: evolution and mechanism

If one were to ask a molecular geneticists why insulin-like growth factor II was imprinted, the answer may well be a statement about the gene having certain features that ensure that it is methylated in one germline but not the other (the precise details are not important here). Ask an evolutionary biologist the same question and a very different form of answer will appear. It may be framed in terms of imprinting being good for this or good for that. How can both answers to the same question be right?

The answer is trivial but important. They are not really the same question. The molecular biologist is interested in the problem of how the imprint is achieved in mechanistic terms. The evolutionary biologist is telling you about why, when the trait was rare (when nearly all *IGF2* alleles were not imprinted), the trait increased in frequency in the population to the point where all (or a significant fraction) of the alleles of *IGF2* are imprinted.

There exists in addition a third form of question. The analysis of the spread of the trait (e.g. an imprinted version of *IGF2*) assumes that at some point in time there were only non-imprinted versions and then somehow an imprinted version appears in the population (initially at very low frequency). How did this come about? This is a question about mutation. This problem has attracted little attention but one

suggestion is worth noting. Barlow (2) notes that viral insertion can induce methylation and hence inactivation of the viral sequences. So, she argues, one might suppose that a mutation occurred converting a gene that did not attract the attentions of antiviral machinery to one that did. If the machinery acted only in one germline and the methyl residues were removed in the other, then this would be a mechanism for the initial creation of an imprint. This attractive suggestion is supported by some data (3). Perhaps many of the mechanistic features of imprinting can be understood from this perspective. The hypothesis does not however explain the spread of the mutation (epigenetic or otherwise) and hence is not in competition with hypotheses for this aspect of the evolution of imprinting. It is the problem of spread that is considered in this chapter.

3. Testing theories of imprinting

Evolutionary hypotheses can be examined on two separate testing grounds. First, one can ask whether in theoretical terms the model is consistent with invasion of the trait and under what circumstances, i.e. can the hypothesis actually account for the existence of the phenomenon? This is the function of much theoretical evolutionary genetics. As we shall see in the next section a few hypotheses are not actually compatible with invasion and should be rejected on these grounds.

Secondly, we can ask whether the hypothesis makes sense of the data. To some degree the adequacy of a theory in these terms is a matter of taste. None the less, certain standards are I think desirable and so I shall 'pass judgement' on the viability and completeness of theories.

4. The facts that should be explained

Before any assessment of the theories is possible it is necessary to identify the facts that need explanation. I start then by identifying the following facts as features of imprinted systems. A theory of imprinting should at least attempt to explain most if not all of these details.

4.1 The comparative data

Imprinting, *sensu stricto*, is found in numerous phylogenetic localities. It is most likely that the phenomenon evolved independently in numerous lineages. It is, for example, reported in at least one unicellular organisms: the chloroplast DNA of the green alga *Chlamydomonas* is methylated dependent upon whether it is derived from + or – type gamete (4). Several parent of origin effects are reported in arthropods. For example, there exists parent-dependent modification of position effect variegation in *Drosophila* (5) and paternal genome inactivation or elimination is found in a variety of arthropods, including sciarid flies, scale insects (coccoids), and mites (6). The most commonly discussed imprinting, however, occurs in mammals and angiosperms. Paternal X inactivity is reported in marsupial soma (7) and eutherian extra-embryonic

tissue (8), whilst gene-specific imprinting is found in eutherians (9) and angiosperms (10).

No one theory is competent to explain all of the above incidences. I shall restrict discussion to theories of imprinting in mammals and angiosperms as these may find a unified explanation (or at least several theories are competent to explain imprinting in these lineages).

One of the failings of several theories is not that they cannot account for imprinting, but that they cannot account for why some lineages do not have imprinting. The lack of imprinting in non-mammalian vertebrates is particularly problematic as they have extensive methylation (see ref. 11). For this reason a constraint type of explanation (i.e. the mutation to produce an imprint is not possible) is not an easily acceptable hypothesis. In addition, the width of the phylogenetic distribution (algae, fungi, higher plants, arthropods, mammals) would suggest that it is not parsimonious to suppose there to be a constraint. Further, that transgenes can be differentially methylated in zebrafish (12) suggests that the establishment of an imprint could be trivial.

4.2 Growth effects

Altered expression levels of imprinted genes, as revealed in for example uniparental disomies, sometimes result in either enhanced or reduced growth of the embryo (see Chapter 7). Comparison of the effects of knock-outs of imprinted genes and non-imprinted genes, shows that imprinted genes affect growth much more often than do non-imprinted ones (13). Imprinting seems also to affect postnatal behaviour, particularly that relevant to suckling behaviour (14) and hence again to growth.

In addition, from a sample of eight imprinted genes known to affect growth, it has been demonstrated that there is a statistically significant correspondence between the direction of growth effects and the direction of imprinting: genes that are paternally expressed tend to be growth promoters, whilst those that are maternally expressed tend to be growth suppressors (15). Since this analysis a further example of a paternally expressed growth promoter has been provided (16). The data used for this test are knock-outs and over-expression studies (see for example refs 17 and 18). *Mash2* may be an exception to this general rule. In agreement with the general pattern is the finding that the invasiveness of placental tissue is positively correlated with the relative proportion of paternally-derived chromosomes (19).

Analysis of uniparental disomies is more ambiguous. Whilst paternal disomies often result in large progeny, and maternal disomies result in the opposite (20, 21), there are several exceptions to these rules (e.g. mouse proximal chromosome 7 and distal chromosome 17) (22).

It is unclear what should be done with data regarding sex chromosomes. That mammalian Y chromosomes have growth factors is consistent with the trend that paternally-derived genes are growth enhancing (23). However, XO mice are larger if the X is maternally-derived than when it is paternally-derived (24) (the opposite of the usual trend). Given that there is probably interference between X inactivation and

this effect, that the XO embryos are retarded at pre-implantation stages (see also Chapter 11), and that XO placental weights tend to be greater than those of XX embryos (25), it is possibly best not to rely too heavily on this data until more is known.

4.3 Multiple genes are imprinted

In eutherians (at least in mice and man) multiple genes are imprinted, not just one (see introductory chapter). Estimates based on the number of CpG islands and the frequencies of those containing imprints (about 6%) suggest that there may be over 100 imprinted genes in mice (26). Several theories of imprinting require only one gene of major effect to be imprinted and hence fail to explain this fact.

4.4 Maternal and paternal imprinting

Several theories explain imprinting in only one germline. These clearly have a problem explaining why there is imprinting in the germline of both sexes in both eutherians and angiosperms. By imprinting in both germlines here I simply mean that some genes are expressed only if paternally-derived and others only if maternally-derived. This, it is known, is not the result of actions in one germline only.

4.5 Not every chromosome has an imprint

In the best studied example, the mouse, it appears that not every chromosome contains an imprinted region. To be more precise (27), we know that in mice, maternal or paternal disomies of some chromosomes effect neither growth nor viability. There are for example probably only six distinct imprinted regions on the 19 autosomes (discussed in ref. 28). It does not follow that there is not an imprint on these chromosomes. However, many theories require that disruption of the dosage of imprinted genes should affect viability and hence, as far as these models are concerned, these chromosomes are not imprinted. The claim that, because there are probably around 100 imprinted genes, it is reasonable to suppose that every chromosome might have an imprint (29) is of uncertain validity as imprinted genes tend to be clustered.

4.6 Lethality

Disruption of the pattern of imprinting has strange effects on lethality. Unbalanced over- and underexpression of some imprinted genes results in lethality (30). In contrast, double deletion of reciprocally imprinted genes does not necessarily result in lethality (e.g. *IGF2, IGF2R*) (31). These two features appear at first sight to be mutually contradictory. As shall be seen however, several models are consistent with this sort of effect, whilst several others are directly falsified.

4.7 Early not late development

Imprinting in mammals and angiosperms is found only in early development (i.e. fetal and postnatal development). For many theories this is a detail that is overlooked and many would predict great advantages if the imprint could be extended into adulthood. That this is not found is hence problematic to these hypotheses.

4.8 Change of status

Imprinting status of particular genes is different in different species. *IGF2R* for example seems not to be imprinted in humans (32) but is in mice (33). That imprinting status might change is a prediction of some hypotheses but not of others.

4.9 Tissue specificity

Many theories can account for imprinting in some but not all tissues. Why, for example, is there imprinting in brain, liver, and placenta? A variety of theories provide explanation of only the latter and hence fail to be convincing. The patterns of X chromosome inactivation are equally curious. Why is paternal X inactivation found in marsupial soma but in eutherian extra-embryonic tissue? By equal measure, can a theory explain random X activity in eutherian somatic tissues. In these cases it is necessary to explain both the pattern and the phylogenetic locality. Claims that it is the maternal genome that maintains the inactive status of the paternal X in extra-embryonic tissues (34) are potentially highly significant but due to conflicting data (35) should not be taken as fact.

4.10 Imprinted genes are not fast evolving

Seven imprinted genes, the sequences of which are available in both mouse and rat, show no lower percentage identity than ordinary (N = 360) genes from the same comparison (Table 1). Imprinted genes are hence not, as a general rule, fast evolving. This result still holds if correction for mutation rate is employed (Fig. 1) (35a). In addition, at their sites of mutual binding both Igf2 and Igf2r are highly conserved (35a). Perhaps these are not surprising results but, as will be discussed, had it been found that imprinted genes are rapidly evolving, one set of theories could have taken this as being strongly supportive.

4.11 Asynchronous replication

Imprinted genes sit in chromosomal domains that have asynchronous replication (reviewed in ref. 36). This may be a side consequence of imprinting (chromosomal domains that are packed / methylated differently may by their very nature replicate at different times). However, a theory that could predict this feature independent of evoking any side consequences must be considered to have some strengths.

Table 1 Percentage identity at the protein level of the seven imprinted genes that have been described in the mouse–rat comparison[a]

Gene	% identity
IGF2	96.7[b]
Insulin 1	93.5
Insulin 2	94.5
Mas-oncogene	97
IGF2R	93.1
Grf1	90.3
SNRPN	100[c]

[a] For this comparison, the mean for an array of 360 genes is ~ 94% ± 8 (SD) (61). Data compiled by the author.
[b] Note also, the guinea-pig sequence is identical to the human sequence (77).
[c] Note, the human sequence is also identical (see ref. 78).

4.12 Chromosomal clustering

Most of the imprinted genes discovered so far are all in one of a few relatively small chromosomal domains. Whilst we cannot be sure that there is no ascertainment bias, what we can be sure of is that some imprinted genes exist in clusters. The fact is important if only because several theories would have predicted that imprinted genes should be maximally dispersed.

There may be a trivial explanation for this clustering, namely, that it is easier to plant an imprint on a gene by extending a locally available imprint. Or perhaps the close location is due to the genes acting as switches (as may be the case for *H19* and *IGF2*). There may however need to be a more sophisticated explanation. In population genetical terms, tight linkage facilitates co-evolution between genes (37) and the clustering may be a consequence of such processes.

5. Theories of imprinting

In broadest outline theories of imprinting can be divided into three categories (for an alternative taxonomy see ref. 38). First, there exists a class that view the phenomenon as an organismic adaptation. Much as the beaks of Darwin's finches are seen as the result of selection to maximize individual fitness, so too imprinting is somehow 'good for the individual'. Often this implicitly means 'good for the mother' and fetal interests are not considered. It may, for example, be a means by which a cell (or progeny) with an abnormal chromosome constitution is guaranteed an early death hence nipping a problem in the bud. An opposing theory sees imprinting as the outcome of a conflict of interests between maternally- and paternally-derived genes. This theory sees selection as underpinning the evolution of imprinting but does not suppose that imprinting is 'good for the individual'. Instead the paternally expressed genes are supposed to be acting as parasites on the maternal supply of resources. The

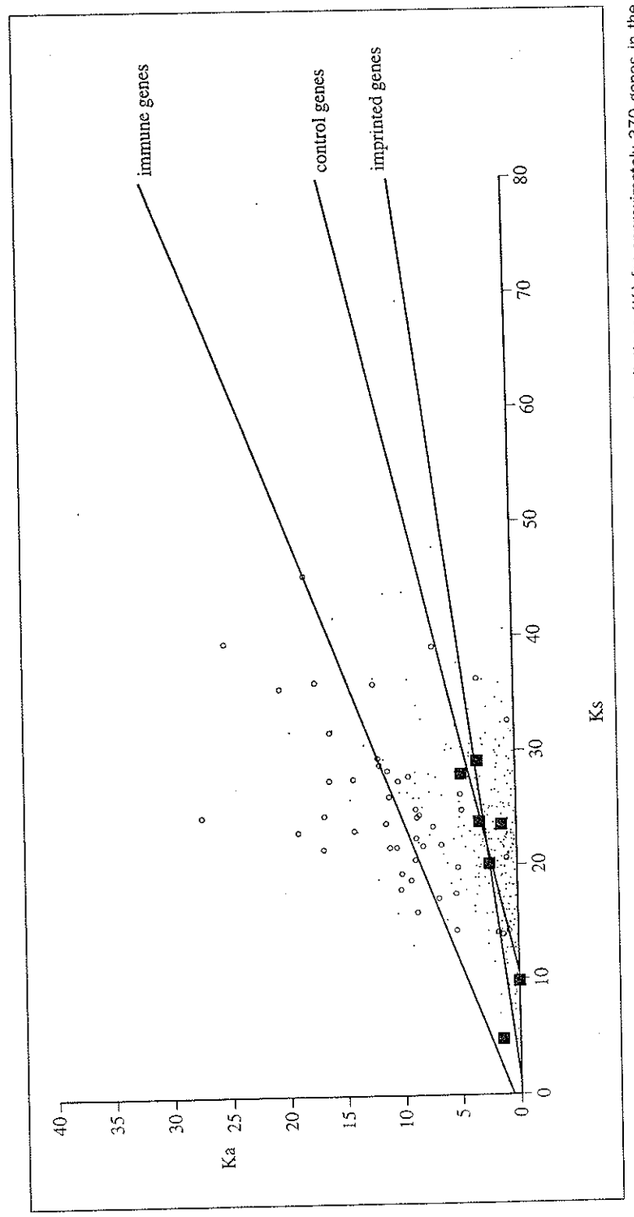

Fig. 1 Plot of the number of non-synonymous substitutions (K_a) against the number of synonymous substitutions (K_s) for approximately 370 genes in the mouse–rat comparison. The genes are split into three groups: immune (circles), control (dots), and imprinted (black squares). The imprinted genes are those given in Table 1. The control set contains a large variety of genes, from receptors to structural proteins etc. Regression lines for each group are given. K_s is typically assumed to be a measure of mutation rate. Statistical analysis reveals that, controlling for mutation rate, immune system genes have a much higher rate of evolution (number of non-synonymous substitutions) than does the control set consistent with antagonistic (i.e. conflict driven) co-evolution. The control set and the imprinted genes are not significantly different. Imprinted genes hence appear not to be evolving in antagonistic arms races as the conflict model might suppose. Data from Wolfe and Sharp (61) and, for imprinted genes from analysis by G. McVean (personal communication).

final set of theories view imprinting as something that is not the product of selection, but rather as a side consequence of evolution.

For want of space I shall concentrate on those theories that have at least some ability to explain the data. I shall not then discuss further the proposition that imprinting could be a means to ensure that the promoter used in one tissue is different from that used in others (39). Although some imprinted genes do have different promoters for different tissues (e.g. *IGF2*) it is hard to explain much more data from this hypothesis. Likewise, the hypothesis that imprinting is involved in antigen switching (40), whilst compatible with the fact that one imprinted gene might have immune function (41), explains little else.

5.1 Imprinting as a result of conflict

Consider a mother that feeds her offspring. Consider also a male who is the father of some, but not all, the brood and that does not feed the progeny. The mother is equally related to all the progeny. The father in contrast is related to its own progeny but not the others. Now consider a father with a novel heritable trait—this male can manipulate the mother and make her provision his offspring more than others. This trait will spread in the population as this male's progeny will on the average be fitter than competing males' progeny. The spread of this trait however creates the context for the spread of a 'resistant' form of mother: if a mother can stop the male from performing this manipulation then this maternal specific trait will spread. This is classical sexual conflict.

Genomic imprinting in the manipulation view is no different from classical sexual conflict, it just notices that the same conflict could be enacted between paternally- and maternally-derived genes within the fetus (14, 21, 42–44). The fetus is hence perceived as a composite of two opposing sets of interests, for, if a mother has more than one mate, paternally-derived genes in any given fetus will not necessarily be related to genes in fellow brood members or subsequent offspring of that same mother. In contrast, a maternally-derived gene in any given fetus has a constant high probability (0.5) that its sibs will contain a clonal copy of it.

The mother would hence 'prefer' to divide resources more-or-less equitably and so maximize her net fitness, not the fitness of any given progeny. In contrast, if the paternally-derived alleles in any given fetus are not going to be present in the sibs, then any decrement in maternal fitness resulting in reduced probability of being able to produce further offspring is, at the very least, irrelevant to them. Paternally-derived genes in a given fetus might thus prefer an allocation of maternally-derived resources in excess of that preferred by the mother. The optimal amount of resources that the maternally-derived genes in the fetus should require will be intermediate between the optima for the paternally-derived genes (a large amount) and the amount the mother should be prepared to provide (a smaller amount). A necessary assumption of this model is that slightly larger progeny will be slightly fitter.

In sum, the expression of genes that encourage the extraction of resources from the mother should be favoured by the paternally-derived genome, whilst the expression

of genes that oppose this to some extent will be favoured by the maternally-derived genome. In the mouse model then, insulin-like growth factor 2 (IGF2) and the IGF2 receptor (alias the cation-independent mannose-6-phosphate receptor) are interpreted as two of the opposing players in this game (43). Fetally expressed IGF2 is one of the factors that are supposed to promote the acquisition of resources from the mother whilst IGF2R, the effect of which is to bind to IGF2 and prevent it from binding to the receptor that will initiate growth, is seen as the response of the maternally-derived genome.

In the above version of the conflict hypothesis, the mother is perceived as that which is manipulated by the paternal genome. It is instructive to note that an alternative version of the conflict hypothesis can be envisaged. One might imagine the mother as making informed decisions as to whom to allocate resources to dependent upon the fitness of the progeny. The intensity of expression of the paternally-derived factors may be an indication of fitness (much as the intensity of begging at the nest by chicks may be an indication of their quality or need) (45). The conflict in these terms can be thought of in terms of the paternal genomes 'desire' to lie about fetal quality and the mother's interest to receive an honest indication of quality. As such a 'signal' hypothesis has yet to be mathematically modelled, it shall not be discussed further, except to note that it seems to make the same predictions as the manipulation hypothesis.

Match to the data—on the whole the manipulation theory provides a respectable match to the data. Perhaps its strongest point is its ability to explain both the existence and direction of the majority of growth effects. The model requires that over-expression of genes that have paternal expression should lead to growth enhancement, whereas over-expression of those genes expressed off the maternally-derived genome should lead to reduced growth. This is the typical pattern.

The distribution of imprinting between tissues (brain, liver, and placenta) is also easily accounted for as the theory is, at one level, one of metabolic manipulation (hence placenta, liver, and hypothalamus), but also potentially one of behavioural interactions postnatally (e.g. one might expect imprinting to affect kin recognition abilities and possibly inter-individual aggression during the postnatal suckling period).

In addition, the theory makes good sense of the phylogenetic distribution (angiosperms, mammals). The hypothesis supposes that intimate contact between mother and offspring is necessary to permit subtle manipulation. Hence, the endosperm–plant boundary, the placental–maternal boundary, and the fetal marsupial–maternal nipple boundary are considered as important to the evolution of imprinting. It should however be noted that this condition is not an absolute prerequisite as postnatal behavioural differences are also considered as being possible grounds for differential activity of genes (see e.g. ref. 14).

Several of the curiosities of the molecular biology of imprinted genes are also explainable. The theory is consistent with the viability of embryos with deletion of both *IGF2* and *IGF2R*. The hypothesis supposes that neither need be absolutely necessary and hence that their absence need not be lethal. Likewise, changes are expected between imprinting systems dependent upon the rate of multiple paternity.

That *IGF2R* is not imprinted in humans may hence be accounted for in terms of the fact that humans are more or less monogamous. It remains to be seen however whether rates of multiple paternity systematically affect imprinting systems as the model predicts. The loss of an imprint at *IGF2R* can alternatively be considered the outcome of a post-fertilization tussle between paternal alleles and *IGF2R* over the control of the latter's imprint status (46).

Genetics conflicts often give rise to arms races between conflicting parties (47–49). In the case of imprinted genes one might expect that this could give rise to selection favouring increased transcription rates. This has been conjectured to explain the intronic paucity of some imprinted genes (15) and the fact that there seem to be more retroposed/intronless imprinted genes than one might expect by chance (15). Whilst the relevance of the data and its interpretation have been questioned (50) it has also been shown that these criticisms were importantly factually incorrect (51).

The theory may also be extended to make sense of X inactivity (14, 52). The paternal X inactivity in muscle in marsupials and in extra-embryonic tissue in eutherians is explained in terms of maternal control of a manipulating X. In the latter case this can be further supported by the finding that it is a maternally-derived gene that maintains the inactive status of the paternal X as required (34). This is a curious observation but given that it could not be confirmed should not be taken as strongly supportive evidence.

A further extension of the theory would suggest that Y chromosomes (being exclusively paternally inherited) should attract growth factors (23). That the mammalian Y does code for growth factors is hence consistent with the hypothesis (23). Likewise, this model predicts (53) that genes avoiding X inactivation should by imprinting-like growth factors, a prediction borne out by the finding of an Xp-linked copy of *Znf127* that avoids X inactivation (see EMBL entry HS4131510). However, the murine Y does not affect net growth (XYY adults are no larger than XY ones), only initial pre-implantation growth rate. In contrast, the human Y has factors affecting adult postnatal growth rates. It may also have genes providing pre-implantation growth effects but this is hard to know.

The manipulation theory is not however perfect. First, it struggles to explain the exceptions to the rule that paternal disomies are large and maternal ones small (although placental size may be large in some of the paternal disomy exceptions). That XO mice are larger when the X is maternally-derived is also potentially problematic but given the interfering factors (see above) this should not be taken as strong counter-evidence.

Secondly, the lethality of uniparental disomies (and related over/underexpression instances) is not a necessary prediction of the model. Indeed, the hypothesis requires that some instances of over-expression must be viable (were this not the case a novel growth promoting paternally expressed allele would never be able to spread in a population). To account for the lethality the theory can evoke co-evolution with the mother, who may, it is argued become adapted to reject 'over-zealous' progeny. Alternatively one might argue that most of the instances of over-expression involve possible doubling of dose and hence exposure to levels of expression never witnessed

in the lineage of any extant mammal. Given also that imprinted genes are clustered most uniparental disomies will also be duplications of several imprinted loci.

Thirdly, the lack of imprinting of *IGF2* type 1 receptor gene (that initiating growth) has been considered problematic. It is however possible to argue that IGF2 type I receptor protein is not limiting in dose and hence a twofold reduction would not affect growth rates. That *IGF1R* (+/–) mice are phenotypically normal, but *IGF1R* (–/–) mice are severly growth retarded, is consistent with hypothesis (54) and hence suggests that the conflict hypothesis has some power to even explain which growth factors might be imprinted and which not.

Fourthly, the theory fails to explain why the soma in mammals has random X inactivation. To explain X inactivation in marsupials the theory notes that maternal–fetal conflict here is all about suckling, so control must be exercised in muscle tissue (14). But eutherians suckle too. Indeed considerable amounts of resources are passed from mother to fetus this way. So why does eutherian soma have random inactivation when marsupials have non-random inactivation? A possible explanation has been provided (52) and it remains to be seen whether the predictions of this model are borne out.

Fifthly, a classical arms race between conflicting parties often may result in rapid sequence evolution of the genes concerned. So, for example, components of the immune system and parasite antigens show dramatically rapid sequence evolution (55–58) (Fig. 1). One might imagine that if the interaction between IGF2 and IGF2R is antagonistic, as supposed, then a gene coding for mutant version of IGF2 that avoided binding with IGF2R should spread (much as a gene for novel parasite antigen spreads). Why then are imprinted genes not fast evolving? Had fast evolution been found this could easily be claimed as evidence in favour of the hypothesis. Slow evolution should hence be considered as being problematic to the hypothesis.

One might argue that the above result is not counter to the expectations of the conflict model if something constrains the evolution of genes involved in maternal–fetal interactions. This argument is somewhat undermined however by the finding that a significant number of genes that are involved in maternal–fetal interactions (59), but of unknown imprinting status, show very high rates of sequence evolution in some lineages: these being placental lactogens 1 and 2 (53, 60, 61), growth hormone (62), growth hormone releasing factor (61), and prolactin (63). A constraint form of explanation does not hence seem valid. However, this latter group of genes are different from most classical imprinted genes as the above tend to be directly involved in transplacental maternal–fetal interactions (i.e. they are either secreted by fetuses into maternal circulation or are the maternal receptors of these factors). The classical imprinted genes tend not to be of this nature and have their activity restricted to fetal tissue. This may be an important distinction. We may then conclude either:

(a) That the second group of genes are involved in maternal–fetal conflict but imprinted genes are not.

(b) That both groups are involved in conflicts but those whose activity is fetal-specific do not evolve fast for some unknown reason.

Sixthly, although not falsified by the findings that imprinted genes have asynchronous replication and are in clusters, no one has yet united these findings with the manipulation theory. This being said however, selection for enhancers of a given effect are expected to provide conditions for selection for linkage disequilibrium (assuming the initial gene is polymorphic). Likewise, imprinted suppressors can more easily spread if tightly linked to a polymorphic imprinted gene with the opposite effect (this author, work in progress).

An intrinsic weakness of the manipulation hypothesis (and indeed of conflict theories of any evolutionary phenomenon (64), not just imprinting) is that it has an inherent flexibility as far as explanation of some of the data is concerned. Consider, for example, the approach to explaining why extra-embryonic tissue in eutherians has paternal X inactivation. The explanation seems reasonable—this tissue is where the paternal X could inflict most damage and so it should be inactivated. What if the facts were reversed? What if the maternal X was inactive in extra-embryonic tissues? An equally 'reasonable' account could be given of this. The extra-embryonic tissues are where the paternal X can have its greatest effects and hence, we would be told, it has a great incentive to dominate and inactivate the maternal X. And what if there were random X inactivation? One could argue that this is simply the default state or perhaps that the two interests are balanced out differently in different cells.

In assessing the validity of the conflict hypothesis then, it is necessary to discount to some extent the ability to explain *post hoc* the patterns of paternal X inactivation. The conflict hypothesis scores over those that cannot explain the facts, but its ability to do so should not be taken as direct support for the theory.

In general, conflict theories are very good at describing the domain of a conflict (e.g. paternal versus maternal genes are in conflict over the control of resources) but, for certain classes of fact, any one of the set of possibilities could be consistent (it all depends on which of the conflicting parties is presumed to be in control). It is better to assess the validity of the theory on the basis of its few risky predictions (such as the direction of growth effects and viability of embryos with only slight over-expression of imprinted genes).

5.2 Imprinting as an adaptation

5.2.1 Imprinting as a defence mechanism

One broad class of hypotheses suppose that imprinting is a defence against one thing or another. Suggestions include, parthenogenesis (65, 66), ovarian trophoblast disease (67), invasive placentas (40), chromosome loss or gain (and hence a variety of cancers, monosomies, and trisomies) (28, 29), dominant disadvantageous somatic mutations, and gametic fusions between same sized gametes (i.e. to stop egg's fusing with eggs and sperm with sperm) (38). This latter possibility, as regards eggs fusing with eggs, is essentially the same as the defence from parthenogenesis argument (at least as applied to mammals) and hence will not be discussed separately.

For these theories to work, it is necessary to suppose that whatever imprinting is a defence against must be deleterious in the short-term. Into a population without

imprinting we may then ask what happens if a modifier of imprinting is introduced, initially at very low frequency. Will this modifier spread? The modifier may spread if the costs of modification (including the increased mortality rate due to exposure of somatic mutations) are less that the advantages accrued from the reduced rate of whatever imprinting is considered as a defence against.

A number of theories (e.g. defence from parthenogenesis, from zygotes with chromosome loss/gain) note that the advantages of aborting unfit embryos are potentially greater in mammals (and by extension angiosperms) as, if done early enough, maternal resources are saved (and can hence be re-invested into relatively fit progeny). This can be contrasted with the state in species in which there is no parental investment after zygotic provisioning. In this circumstance there is little or nothing to be gained from aborting unfit embryos as this does not free up unused resources (here I assume a negligible rate of inbreeding and an absence of sib competition, the avoidance of both of which can provide advantages to the abortion of progeny even in species with no parental resource allocation). This feature of these theories is attractive in so much as they go some way to explaining the phylogenetic distribution (see later).

i. Parthenogenesis

Whether a trait providing a defence against parthenogenesis could invade a population is not as straightforward an issue as might at first be thought. One might imagine that as parthenogenetic lineages tend to go extinct, so a means to prevent the initiation of a doomed lineage would be a good idea. Imagine, however, that the occasional female is incapable of sexual reproduction and hence can only reproduce asexually. Imprinting would probably prevent her from doing this. But why should the death of these parthenogenetic embryos allow the spread of a gene whose function is to kill these progeny? The gene dies in the progeny as well.

A modifier of imprinting could however potentially spread if females produced occasional parthenogenetic progeny. The death of these could, through re-allocation of resources, provide benefit to surviving sexual progeny of the same mother, some of whom will contain the modifier. Even this however is not an adequate condition. If the potentially asexually produced progeny were capable of sexual reproduction then a female may be better off producing parthenogenetic progeny than aborting the asexually produced ones.

Alternatively, if the asexual progeny were to die then early abortion would indeed be a viable strategy. In this instance, the model requires that imprinting acts to force abortion prior to the termination of maternal investment and prior to the time that natural mortality (perhaps due to homozygosity) would occur.

Match to the data—the defence from parthenogenesis arguments has two strong features. First, it is consistent with the phylogenetic distribution. Necessary to the argument is reproductive compensation and hence species with continuous provisioning of young (marsupials, eutherians, and angiosperms) are those that are expected to have imprinting. Secondly, it is consistent with the fact that imprinting appears to be reduced when provisioning stops.

In its favour is also the finding that there are no parthenogenetic mammals. In this regard however, the hypothesis has a problem with the existence of parthenogenetically-derived angiosperms (21). That imprinting is restricted to endosperm has been taken as evidence in favour of the conflict hypothesis and against the prevention of parthenogenesis theory (44). The greatest weakness of the hypothesis however is that it cannot explain why imprinting occurs in both sexes (inactivity of paternally-derived genes is unnecessary) and hence cannot account for the correspondence between the direction of growth effects and the direction of imprinting. Similarly, paternal X inactivation in marsupials and eutherians is unaccounted for. In addition, whilst the theory is competent to explain the inviability of most uniparental disomies, the parthenogenesis hypothesis fails to explain the viability of mice with deletions of *IGF2* and *IGF2R*. It is also unclear why more than one gene need be imprinted. An imprint on one gene vital for early development would be adequate. The hypothesis also struggles to account for a turnover of imprinting status.

In general when the theory succeeds it does so on those points that are also the strong points of the conflict hypothesis. To my mind, however, it leaves too much unexplained to be considered a complete theory of imprinting.

ii. Ovarian trophoblast disease and invasive placentas

Mammalian fetuses are quite aggressive invasive things. One would not want one burrowing into tissue that was not prepared for it, nor burrowing too 'aggressively' into tissues that are prepared. Hence, Varmuza and Mann (67) argued that, by shutting off genes in the maternal germline that are necessary for early embryonic growth, ovarian trophoblast disease is avoided (67). Similarly Hall (40) notes that imprinting may restrain aggressive placentas from harming a mother. The former theory may be positioned as a subdivision of the defence from parthenogenesis argument (68). It is however different from the usual formulation of this argument in so much as the cost that parthenogens inflict is, in this model, made explicit.

Assuming that the frequency of ovarian trophoblast disease in the absence of imprinting is adequate to account for the costs of imprinting, then, at least on theoretical grounds this theory is fine. It has however been argued that the incidence of ovarian teratomas is very low in non-humans and hence for most mammals this is not a problem (68). The data is uncertain (69).

Match to the data—these hypotheses are consistent with the fact that the genes inactivated in maternal germline are frequently growth promoters, often with placental activity. It would hence be strange if imprinting did not reduce to some degree the incidences of ovarian trophoblast disease (see also ref. 70). The problem with these arguments, as with parthenogenesis arguments in general, is that they leave too much unexplained to be considered complete explanations (for discussion see refs 71 and 72).

The hypotheses account for a more restricted amount of the phylogenetic dispersion than the parthenogenesis hypothesis. Whilst it can be argued that eutherians are unusual in having a problem with invasive trophoblasts and hence need imprinting, the hypotheses do not make sense of imprinting in angiosperms

which do not have invasive trophoblasts (71, 72). The same might be said of marsupials. This point Varmuza and Mann (69) object to arguing that it is unknown if imprinting in angiosperms and eutherians has one origin (and hence if not they may not be comparable). This is an irrelevant reply however as the criticism concerns the phenotype of imprinting, not the mechanistic means by which this is achieved.

Like the other parthenogenesis arguments, these two defence hypotheses do not convincingly explain why any imprint should ever leave a paternally-derived gene inactive (and hence also fail to account for X inactivation patterns) and hence why these should be growth repressors. In addition they also fail to explain imprinting activity in most tissues (e.g. brain and liver) and why the imprinting status persists until after birth. As regards the ovarian teratoma defence hypothesis, it is again unclear why more than one gene need be imprinted (71). One gene that initiates zygote development that is switched on only after being paternally-derived would be perfectly adequate.

There are many other details that, along with the parthenogenesis argument, these hypotheses make no sense of: asymmetric replication, chromosomal clustering, loss of imprinting at *IGF2R*. Whilst no doubt some proportion of the selective deaths favouring imprinting are the result of a higher rate of trophoblast disease in non-imprinted competing lineages (71), and no doubt mothers do benefit from not having over-invasive embryos, in general, these hypotheses cannot be considered thorough explanations of imprinting. Haig and Trivers (73) have proposed that a simple way to show the above two theories to be non-general explanations would be to show that mammals with non-invasive placentas also have imprinting. This has yet to be done.

iii. Chromosome loss and gain

The theories for chromosome loss/gain can be divided on two axes (all possible theories can hence fit in one of four spaces in a two by two square). First, one set of theories sees imprinted genes as necessary, whilst another set sees them as toxic. Secondly, one set envisages the advantages of imprinting as coming about due to mortality of cells that have somatic mutation in chromosome number (this could possibly occur in adult life), whereas another set sees the advantage being solely due to inherited chromosomal abnormalities and acts to affect fetal viability. If one evokes somatic abnormalities that occur soon after the first zygotic divisions, then the two hypotheses collapse to the same. It is however helpful to separate out those theories that rely solely on fetal viability effects from those that need have no impact on fetal viability.

If imprinted genes are necessary genes, the loss of whose activity is fatal, the loss of a chromosome may then cause cell lethality (and hence possibly protect against cancer) (28) or fetal lethality if the monosomy was inherited (28; see also ref. 29). Monosomics are however inviable without imprinting. For this to function as a theory of imprinting lethality should occur (as with the parthenogenesis argument) sooner than in the non-imprinted case. This advantage is open to experimental verification.

The alternative to the necessary gene argument is to postulate that the products of imprinted genes are toxic. Thomas (29) argues that reciprocally imprinted genes may

be titrated against each other and the toxic effects of both effectively annulled. This theory is then claimed as a defence from both chromosome loss (as with the necessary gene argument) but also chromosome gain as both conditions affect the balance of the titrated products.

Thomas notes that with imprinted genes the titre doubles on chromosome gain, whereas with non-imprinted genes it goes up by a factor of 1.5. Hence, he suggests, the lethality is affected sooner than it would be were the organism functionally diploid at the locus concerned. However, this argument as presented in terms of the proportional increase in gene products can be misleading, as in both the imprinted and the non-imprinted cases the actual increase in the amount of product is the same, if the haploid level of expression is the same.

Consider that if the gene is imprinted it is expressed at a rate x but if it has diploid expression it is expressed at a rate 2x (this would seem a reasonable assumption at the point of origin of the imprint status). What then happens in a trisomic? If the additional chromosome does not contain an active imprinted gene there will obviously be no difference in titre. If it does contain an active gene, the dose will go up from rate x to rate 2x, a difference of x. But the same difference is found even if the chromosome is not imprinted. Under this circumstance the rate will go up from 2x (the diploid rate) to 3x. What is more, if an organism has diploid expression, this increment occurs in all trisomics. Hence, assuming the reciprocal genes were balancing each other's toxic effects (the reciprocal gene being at effective titre x in the imprinted genome and titre 2x in the non-imprinted one), the net excess dose of toxin is the same for both imprinted and non-imprinted instances (i.e. x). Imprinting in this model is of no advantage and if all chromosomes are not imprinted would even be a disadvantage when compared with the diploid model! The same is true in the case of chromosome loss.

Furthermore, under Thomas's version of the model, the invasion of the trait is impossible. The invasion of a gene coding for a toxin is, not unsurprisingly, hard to envisage. If the products really are toxic then one must suppose that the two mutually cancelling toxins should arrive in the population at the same time, and in the same genome. If one arrives before the other, selection will immediately oppose it (it is always in a dead progeny!). Further, the two must perfectly balance each other out from the outset and all individuals with a paternal imprint must also receive the reciprocal maternal imprint. When the trait is polymorphic it is very hard to see how this might occur when the two genes are unlinked (as is the case with *IGF2* and *IGF2R*). In sum, this theory should be rejected on theoretical grounds.

Match to the data—as discussed above, the chromosome loss/gain hypotheses can be one of four varieties. The strongest are those based on early abortion of fetuses as these are consistent with the phylogenetic distribution. As with the parthenogenesis argument, these may explain the correlation in terms of the saving of resources if death of the embryo is adequately early. This set of theories is also compatible with the fetal specificity of imprinting.

In contrast, the cancer protection/somatic viability argument does not account for the phylogenetic pattern (why do not other vertebrates do it?) and would predict that

imprinting should extend through to maturity. The cancer protection hypothesis would also not predict imprinting in non-dividing cells such as brain cells and has a problem explaining the lack of a relationship between monosomy and cancer (discussed in ref. 29).

As regards the other axis, the necessary gene model and the titration of toxins models can make some sense of some data but are contradicted by more. Unlike the necessary gene model the toxin model has the advantage of being able to explain the viability of *IGF2/IGF2R* null mice. Given the inadequacy of this hypothesis on so many counts, however, this finding should not be taken as license to consider this hypothesis seriously. The necessary gene model would appear to be falsified by this finding.

Both sets of theories whilst not predicting that imprinted genes should effect growth are not falsified by the fact. Neither set of hypotheses predicts the covariance of growth direction and imprint direction and this should be considered a major weakness.

In contrast to the above two models (parthenogenesis and ovarian trophoblast disease) the loss/gain models are consistent with an imprint in both males and females and the existence of multiple imprinted genes is what would be expected. This being said however, both forms of the hypothesis struggle to explain why uniparental disomies of only a few parts of mouse chromosomes affect fitness. The theories would predict extensive spacing of imprinted genes so as to ensure one on every chromosome. Why have two paternally imprinted genes in a very small domain? Thomas, in advocacy of the loss/gain arguments notes that there are probably over 100 imprinted genes and supposes that it is reasonable to argue that every chromosome will have one. However, as noted previously, the uniparental disomy data suggests that even if all chromosomes do have such genes they are not effective when lost, at least as regards fetal abortion (as would be required by the model). Secondly, as it is well known that imprinted genes exist in clusters, it is unclear that it need be the case that imprinted genes exist on every chromosome. In summary then, none of the version of the chromosome loss/gain arguments make particularly good explanation of the facts.

iv. Dominant deleterious somatic mutations

That imprinting affects the dominance of mutant versions of the genes involved must be true. It is unclear whether this could however be the reason for the spread and maintenance of imprinting (27). The idea that it might be has rightly been rejected by Sapienza on the grounds that imprinting would be disadvantageous as it would ensure that deleterious recessives would be expressed and dominant gain of function mutations would be hidden when inactivated (27). Whilst this is true, it suggests the conditions under which imprinting, as a means to alter dominance might work. By extension of Sapienza's logic, imprinting would be a good defence against dominant deleterious somatic mutations and increase the probability of exposure of advantageous recessives (the case for non-somatic mutations is more complicated and will not be considered here).

In a diploid organism a dominant mutation could occur on either chromosome and cause deleterious effects. However, with imprinting, approximately half the time the mutation would affect the silent copy (no fitness reduction) and half the time it would affect the active copy. In the latter case, the fitness reduction would be the same as that in the diploid example (assuming the mutation to be of full penetrance).

Conversely, if the mutations that affect imprinted genes are advantageous recessives then again, imprinting can be favoured as these recessives then stand a better chance of being exposed before random drift removes them from the population. This latter form of the argument is not a defence argument but due to its relationship to the defence argument it is appropriate to include it here.

A further slight twist can be put onto these arguments by noting that the model becomes yet more robust either if the penetrance of a mutation is positively correlated with the degree of fitness reduction (or, in the advantageous inverse case, if the degree of fitness increment is negatively correlated with penetrance). If the fitness consequences of mutations affecting a gene were such that highly penetrant ones were very deleterious, but highly recessive ones were greatly advantageous then somatic imprinting of such loci would be highly advantageous (if the mutation rate were adequately high).

Match to the data—this theory has only limited ability to explain the data. It is consistent with the fact that numerous genes are imprinted and that not all chromosomes have an imprint with major fitness effects. However, although it explains imprinting, it does not really account for the phylogenetic distribution and, like so many other hypotheses, it fails to allow one to understand why every diploid organism doesn't have imprinting. In addition, the fact that imprinting stops soon after birth is not what would have been predicted (in this regard this theory is quite similar to chromosome loss/gain models of somatic viability). The direction of imprinting and possible relationship to growth effects are not inconsistent but would not have been predicted.

The assumption of the model that some genes are more liable to be affected by dominant deleterious mutations or advantageous recessives is also very questionable, although one might appeal to the instance of p53 as regards deleterious dominants. Mutant versions of this gene, when co-translated with wild-type versions appear to alter the wild-type protein to behave as if mutant type. This is a novel form of dominance. One might however wonder why p53 is not imprinted. Going against the hypothesis however is the finding that typically very recessive genes tend to be very deleterious (discussed in ref. 74)—the opposite of what the model requires. By equal measure however, one might then claim that genes that are different in this regard are going to be quite rare and hence there should only be a few imprinted genes in the genome.

Advantageous mutations are also thought to be rare so a model predicated on their arrival in the soma is on relatively weak ground, but again the same logic concerning the rarity of imprinted genes could be employed (perhaps these do have a high somatic mutation rate and one where most changes are advantageous). In this regard however, that imprinted genes appear not to be rapidly evolving (Table 1) can be considered as contrary to the expectation of this hypothesis. This all being said, the effect of mutation on imprinted genes is open to verification, but given the limitations of this hypothesis, this should possibly not be granted high priority.

5.2.2 Gene regulation

i. Minimizing variance in rates of expression

Imprinting may be a means to allow accurate control of genes and so minimize variance in rates of growth of progeny (66). At least two reasons for this can be envisaged. First, consider that there was noise in the control of gene transcription and hence that the accuracy of control was not constant—it may be easier to down-regulate expression of a gene by one-half than by three-quarters. If this is so, and if complete inactivation is easy, then if a gene is needed at low dose it may be more efficient to shut off one gene and down-regulate another by one-half, rather than down regulate two by three-quarters. Secondly, if a gene product is required at high doses but with minimal variance in expression levels, then, by ensuring that only one allele is employed, transcription may approach some limiting rate. When there is an excess of transcription factor the variance in transcription rates would be minimal. The same variance minimizing effect can however be produced by having diploid expression and an even higher level of transcription factor. This would however result in twice the level of product which need not be advantageous. It is the case that imprinting is not required for such control of dosage. It is however one potential means.

The theory as presented above is however unconvincing as it does not account for why everything does not have such regulation. To take account of this Solter (66) restricts this model to consideration of a brief period of eutherian growth. The importance of imprinting in Solter's model is that large progeny make the fitness of mother and other progeny lower. In effect, he argues, the variance in growth rates is amplified as regards fitness consequences in eutherians. Solter's restriction of the model to consideration of a brief period of development in eutherians is not necessary. Any variance in resource uptake and interference between the progeny of a mother would be under the same selection. Hence it need not be restricted to eutherians but to any species with adjustable parental investment. It is this slightly extended version that is the most competent to explain the data and hence I shall consider only this model.

Match to the data—an interesting property of this slightly extended model is that it makes nearly precisely the same set of predictions as the conflict hypothesis. Rather than being a theory about conflicts over resource extraction, it is instead more about co-operation. Its presumption is that progeny are so highly related that they should co-operate and not extract too many resources. Any variance in resource extraction should be minimized and imprinting is a viable means to do this. In this regard this theory is compatible with imprinting in endosperm rather than progeny. Likewise, the phylogenetic distribution of imprinting (mammals and angiosperms) is accounted for (these species all have the potential amplification of effects due to skews in resource distribution). That imprinting does not occur in mature individuals

is what would be expected and the tissue specificity is understandable. If the conflict argument can explain these tissues in terms of competition between brood members, then one can suppose that reduced variance is also a viable strategy.

Likewise, the fact that multiple genes are imprinted is consistent, as is the finding that, at least in mice, not every chromosome has an imprint that affects fetal viability. That some genes can alter their imprinting status (e.g. *IGF2R* is not imprinted in humans) can be accommodated.

The hypothesis can explain why over-expression of imprinted genes so frequently results in inviability. One can simply postulate maternal rejection of over- or undersized progeny. It is also consistent with slow evolution of imprinted genes.

The argument however does not make sense of some data. Although the model predicts that imprinted genes should be associated with growth, the model cannot easily accommodate the direction of such effects. The model would not predict that paternally expressed genes are growth enhancing but maternally expressed ones are the opposite. Furthermore, the hypothesis similarly does not require that *IGF2R* and *IGF2* need be imprinted in different germlines. Indeed in general, the hypothesis requires an imprint only in one germline. Perhaps most importantly, if variance between progeny is to be reduced there should never be paternal expression as most of the variance between progeny is paternally derived (if there is polyandry). This being said, the hypothesis can then make good interpretation of the paternal X inactivity in what the conflict hypothesis sees as the tissues that matter. By equal measure, if this is to be argued, then the argument must have similar difficulties as the conflict hypothesis explaining random X inactivation in eutherian soma.

Like the conflict hypothesis this one cannot explain why imprinted genes are associated with chromosomal regions with asynchronous replication, why they are clustered and why IGF2s growth promoting receptor is not imprinted. The theory as formulated above does not attempt to account for imprinting in arthropods, yeast, or *Chlamydomonas*, although there may be other situations in which variance in gene regulation is important. Likewise, the finding of imprints not affecting growth need not be inconsistent.

ii. Control of cellular differentiation

Holliday (75) has noted that the control of cellular differentiation is much more regular with imprinting than with diploid expression. He considers the problem to be one of segregation of inactive and active status at mitosis (see Fig. 2). Consider, for example, a stem cell that must divide to produce both another stem cell and also a cell that will become specifically differentiated. In such a cell, Holliday argues, there is a segregation of gene activities during cell division. Assume, as seems reasonable, that a change in a stem cell to become differentiated involves some molecular level switch (methylation for example) that occurs during chromosome replication. Pre-mitotically there will then be a pair of centromeres each with two chromatids. A cell that initially had two genes in active status may then inactivate one copy during DNA replication such that within each chromatid pair one copy of the gene is active and one inactive. The problems arise because mitosis is a random event. Hence either both

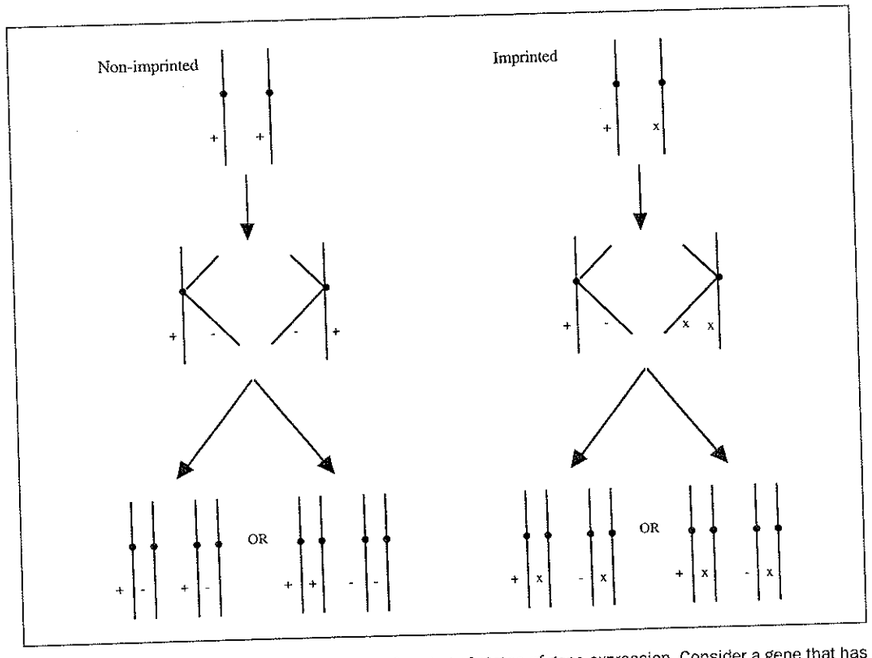

Fig. 2 Holliday's theory of imprinting—accuracy of control of states of gene expression. Consider a gene that has three possible states, permanently locked (i.e. cannot switch state) (x), active (+), and inactive (–). It is desirable to have a mechanism that ensures segregation of activity status such that one cell can always, if needs be, produce two daughter cells in different configurations, whilst also conserving the original condition (e.g. a stem cell generating another stem cell plus a differentiated lineage). This is problematic if the initial condition is diploid expression (i.e. non-imprinted). Consider that the cell starts off with both copies active. Switching occurs during DNA replication so the pre-mitotic cell has two identical chromatid pairings. At mitosis however, one of two segregations is possible. Either both active copies go to one cell and both inactives to the other (as needed for differentiation between cells) or both cells have one active copy and one inactive one. If however, one of the two copies starts permanently locked and hence cannot switch (i.e. with imprinting) then the daughter cell combinations are always the same. One contains an active plus a locked chromosome, the other has an inactive and a locked chromosome. Note this is not necessarily a theory of imprinting and could be one of random allelic exclusion (compare with random X inactivity).

cells will end up with one chromosome with the gene inactive and one with the gene active, or, one cell will inherit both active genes and the other the pair of inactive genes. In the first case there is no difference between the daughter cells and hence both must be in the same state.

The complication will not arise if one of the diploid pair of chromosomes always maintains its gene inactive. At the mitosis, one cell will receive one active and one inactive copy and one cell will receive two inactive copies. Inactivation of one of a pair of developmentally important switch genes thus ensures tighter control of cell

differentiation. This theory is not really a theory of imprinting in so much as the model requires simply that one allele be inactive and random inactivation would be adequate.

Match to the data—the theory has problems with several observations. First, as with the variance in rates argument, although growth effects are consistent with the model, the direction of growth effects is not expected. Secondly, Holliday predicts that imprinted genes must be vital to development. Whilst supported by the inviability of many uniparental disomies, the model is falsified by the finding of the viability of double deletion mice. Thirdly, the model envisages random X inactivation as being equivalent to non-random inactivation. Why then do some tissues have non-random X inactivation but others have random inactivation? Fourthly, the model is one of the class that have a problem explaining why every organism does not have imprinting. One must be tempted to ask how it is that any multicellular organism could ever develop without allelic exclusion of some variety. Fifthly, the model does not require that both germlines need be imprinted (and imprinting in only one would seem more parsimonious). The model is however consistent with there being multiple loci that are imprinted and with the slow evolution of imprinted loci. In general, however, this does not seem an especially convincing argument.

iii. Temporal spacing

A further variety of theories relate imprinting to control of gene expression (40). It could, for example, be thought that imprinted genes are so vital that during replication transcription is necessary so imprinting is a means to ensure that one copy replicates early and the other one late, so guaranteeing that replication does not interfere with transcription.

Match to the data—the temporal spacing argument, although adequate in theoretical terms, has many failings. The phylogenetic distribution is not accounted for, the direction and existence of growth effects are not predicted, and the viability of *IGF2/IGF2R* null mice is not what would have been expected. The pattern of X inactivity in marsupials and eutherians is unaccounted for and it is unclear why non-dividing brain tissue should have imprinting. That imprints do not extend to maturity is also not well accounted for.

One can also imagine easier ways to achieve the same effect—one might for example have diploid expression and translational control of gene expression, as done for example in many late translating genes in spermatozoa (76).

This all being said, there may be something in the argument as it is found that imprinted genes are in the chromosomal domains for which the time of replication is asynchronous (reviewed in ref. 36). This however is not a unique feature of imprinted genes and may simply reflect conformational differences in methylated and unmethylated sequences.

This author is also unaware of any evidence that the 'inactive' imprinted allele is ever active during the replication (and transcriptional inactivity) of the other allele. Until there is concrete evidence that inactive alleles are active when the active ones are being replicated, this hypothesis sits on weak territory.

5.3 A side-product

A few authors have conjectured that imprinting may simply be a side-product (see e.g. ref. 38). Both the view that imprinting is a defence against parthenogenesis and a defence against ovarian teratomas must account for the inactivity of some paternally-derived genes as a curious side-effect. Is this adequate? Solter (68) (one of the proponents of the parthenogenesis hypothesis) argues that 'we should first try harder to explain all imprinted phenomena in these [selective] terms before we give up and resort to the idea of the innocent bystander'. I can only concur.

In contrast, it is probably quite reasonable to suppose that differences in the age of onset of various diseases, dependent upon the sex-specificity of the inheritance of the allele, is not the product of selection and hence a side-consequence. The difference between these disease onset findings and 'normal' functioning of imprinted genes is the within population rarity of the former (and possibly their restriction to within humans). That is not to say that an adaptive explanation cannot be sought, it is just not obvious that one is needed.

6. Conclusions

No theory of imprinting can explain all incidences of imprinting. The examples in arthropods and imprinting of chloroplast DNA in *Chlamydomonas* are best considered as separate phenomena (but see discussion of accurate control given above). Theories do however exist that can potentially put imprinting in eutherians, marsupials, and angiosperms under one roof.

A few theories can be discounted as being logically inviable (e.g. the titration/toxin model of chromosome loss). However, most theories, even if logically viable, do not stand up well when scrutinized against the data. Most are not competent to make parsimonious interpretation of the facts, for example the phylogenetic distribution, the growth effects, and fetal specificity.

Two theories (the conflict hypothesis and the minimization of variance in rates of expression hypothesis) stand out in that they can make sense of many of the facts. Both have in common the view that imprinting is related to the control of embryonic growth. Both hypotheses have trouble explaining patterns of X inactivation (but see ref. 52). Neither claims to explain the clustering of imprinted genes (but see above) or their asynchronous replication.

The variance argument scores over the conflict hypotheses in being consistent with the fact that imprinted genes are not relatively fast evolving. This finding does not however falsify the conflict model. More critically, the conflict arguments scores over the control argument as regards prediction of the correspondence between the direction of growth effects and the direction of imprinting. The conflict hypotheses suppose that over-expression of paternally-derived alleles should give growth enhancement. The accurate control hypothesis predicts either a random pattern, or that paternally expressed genes should never be active. As this correspondence between growth direction and imprint direction was the one discriminating risky

prediction that the conflict argument makes, it appears that the conflict model at present provides the most convincing match to the data. It remains to be discovered whether this pattern is highly robust and why, when it comes to uniparental disomies, there exist some exceptions to the rules.

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