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Parental modifiers, antisense transcripts and loss of imprinting

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The kinship theory of genomic imprinting has explained parent-specific gene expression as the outcome of an evolutionary conflict between the two alleles at a diploid locus of an offspring over how much to demand from parents. Previous models have predicted that maternally derived (madumnal) alleles will be silent at demand-enhancing loci, while paternally derived (padumnal) alleles will be silent at demand-suppressing loci, but these models have not considered the evolution of *trans*-acting modifiers that are expressed in parents and influence imprinted expression in offspring. We show that such modifiers will sometimes be selected to reactivate the silent padumnal allele at a demand-suppressing locus but will not be selected to reactivate the silent madumnal allele at a demand-enhancing locus. Therefore, imprinting of demand-suppressing loci is predicted to be less evolutionarily stable than imprinting of demand-enhancing loci.

Keywords: genomic imprinting; antisense; demethylation; evolutionarily stable strategy; *IGF2R*; modifier

1. INTRODUCTION

Genomic imprinting is the phenomenon whereby a gene's pattern of expression depends on its parental origin. The kinship theory of genomic imprinting (Trivers & Burt 1999) attributes the evolution of imprinting to a conflict of interest between the two alleles at a locus in a diploid individual. Specifically, natural selection favours a pattern of expression that increases the individual's matrilineal inclusive fitness when the allele is maternally derived, but favours expression that increases the individual's patrilineal inclusive fitness when the allele is inherited from the individual's father (Haig 2000).

Imprints are established in parents but interpreted in offspring. We will use the adjectives 'madumnal' and 'padumnal' to refer to alleles that an individual inherits from their mother and father, respectively (Haig 1996). These terms distinguish maternally and paternally derived alleles that are expressed in offspring (madumnal and padumnal alleles) from alleles that are expressed in mothers and fathers (maternal and paternal alleles). This distinction is important because the selective forces acting on madumnal alleles differ from those acting on maternal alleles, and likewise for padumnal and paternal alleles (Haig 1992; Burt & Trivers 1998).

Earlier models have predicted silencing of madumnal alleles at demand-enhancing loci and silencing of padumnal alleles at demand-suppressing loci (Haig & Wilkins 2000; Kondoh & Higashi 2000; Wilkins & Haig 2001). In these models, natural selection chose an unbeatable strategy from among a set of all pairs of non-negative values, $\{x_m, x_p\}$, where each pair represented a different combination of levels of madumnal and padumnal expression. Thus, these models assumed that expression levels at evolutionary equilibrium could be modelled simply by considering selection as acting on alternative alleles at the imprinted locus itself, without considering selection on *trans*-acting modifiers expressed in parents. That is, genes expressed in parents were implicitly assumed to be unable to impose patterns of expression on genes expressed in offspring that act against the latter genes' interests. Burt & Trivers (1998) identified additional conflicts that are possible if *trans*-acting factors are capable of influencing patterns of imprinted expression. They point out that natural selection will act differently on the imprinted genes, *trans*-acting factors expressed in offspring and *trans*-acting factors expressed in parents, potentially resulting in a conflict of interest between different components of the imprinting machinery.

The importance of the actions of genes expressed in parents in determining patterns of imprinted expression has been brought into focus by a recent review of the apparent mechanism of imprinting at 17 imprinted loci. Reik & Walter (2001*a*,*b*) found a striking asymmetry in the means by which madumnal and padumnal alleles are silenced. At 10 out of 10 madumnally silent loci, the madumnal allele is silenced by direct methylation of its promoter. By contrast, at five out of the seven padumnally silent loci, the padumnal allele is silenced indirectly by methylation of the madumnal promoter of a (padumnally expressed) antisense transcript. Thus, in 15 out of 17 cases, imprinting is achieved by methylation of a madumnal promoter. At most padumnally silent loci, the straightforward mechanism of repressing a padumnal promoter is avoided in favour of an indirect mechanism of repressing the madumnal allele of a *cis*-acting repressor.

Reik & Walter (2001*b*) suggested that this asymmetry could be explained by the genome-wide demethylation of the padumnal genome that occurs in mice immediately after fertilization (Mayer *et al.* 2000; Oswald *et al.* 2000). In their view, 'demethylation of the paternal genome can be viewed as a reprogramming mechanism by which the

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egg… strips off paternal imprints when the paternal genome is at its most vulnerable' (p. 255). Thus, they assume that demethylation of the padumnal genome is determined by maternal factors present in the egg cytoplasm, rather than by paternal factors entering with the sperm.

In the present paper, we will expand our previous analyses of conflicts between madumnal and padumnal alleles at a demand-enhancing (or inhibiting) locus expressed in offspring to include a second locus that is expressed in a parent and whose products act in *trans* to determine the maintenance of epigenetic marks in offspring. An allele's level of expression at the primary locus will be viewed as jointly determined by an intrinsic expression level (encoded in the allele's DNA sequence) that is independent of parental origin, and by epigenetic modifications of this sequence. The latter will be viewed as determined by interactions between *cis*-acting elements in the allele's own sequence and *trans*-acting factors expressed in the parent. The strategies available to alleles at the primary locus will be expanded to include the production of an antisense transcript that represses the production of sense transcripts in *cis*. Antisense transcription is also subject to epigenetic modification by alleles at the parental modifier locus.

2. FITNESS FUNCTIONS

At the primary locus, we consider a gene whose quantitative level of expression influences the distribution of maternal resources among offspring. The total level of expression X is the sum of x_m, expression from the madumnal allele and x_p, expression from the padumnal allele (where x_m, $x_p \geqslant 0$).

The fitness of the current offspring is represented by U and the aggregate fitness of the mother's other offspring by V. The fitness W_{mp} of a rare allele present in the current offspring is the average of the offspring's matrilineal inclusive fitness when the allele is maternally derived and the offspring's patrilineal inclusive fitness when the allele is paternally derived (see Haig 1997, 2000):

$$W_{mp} = (W_m + W_p)/2. \tag{2.1}$$

Matrilineal and patrilineal inclusive fitness are given equal weight in this average because, over the course of many generations, an autosomal allele will be maternally derived half of the time and paternally derived half of the time.

An evolutionarily stable strategy (ESS) is defined as a pattern of expression that, when adopted by most alleles in a population, is resistant to invasion by rare alternative strategies. That is, an ESS constitutes a local fitness maximum. Thus, we will focus on the effects of natural selection on rare alleles with expression strategies that differ by a small amount from an established strategy that is near fixation in the population.

At an imprinted locus, we will assume that changes in x_m are independent of changes in x_p. Therefore, the effects of small changes in madumnal or padumnal expression on an allele's fitness are given by:

$$\frac{\partial W_{mp}}{\partial x_m} = \frac{\partial W_m}{\partial X} = \frac{\partial U}{\partial X} + \frac{1}{2}\frac{\partial V}{\partial X}, \tag{2.2}$$

$$\frac{\partial W_{mp}}{\partial x_p} = \frac{\partial W_p}{\partial X} = \frac{\partial U}{\partial X} + \frac{k}{2}\frac{\partial V}{\partial X}. \tag{2.3}$$

The factor of one-half in equation (2.2) represents the expectation that a rare madumnal allele present in a given offspring will be present in half of the individual's mother's other offspring. By contrast, a rare padumnal allele is expected to be present in a fraction $k/2$ of the mother's other offspring, where k represents the proportion of the mother's residual reproductive value that is shared with the offspring's father. We will assume that $0 \leqslant k < 1$. The factor k is equivalent to $2p$ in our previous models (Haig 1996; Wilkins & Haig 2001) and is used here to make these results more easily compared with those of Burt & Trivers (1998).

If all alleles were subject to the constraint that their expression is independent of parental origin ($x_m = x_p = x_{mp}$), then the locus would be unimprinted and an allele would have the same effect on X when maternally or paternally derived. Thus, the effect of natural selection on a rare allele would be simply the average of its effects in these two circumstances:

$$\frac{\partial W_{mp}}{\partial x_{mp}} = \frac{1}{2}\left(\frac{\partial W_m}{\partial X} + \frac{\partial W_p}{\partial X}\right) = \frac{\partial U}{\partial X} + \left(\frac{1+k}{4}\right)\frac{\partial V}{\partial X}. \tag{2.4}$$

Alleles at a maternal modifier locus are assumed to act in *trans* to erase epigenetic marks that silence alleles at the primary locus. Therefore, the change in maternal fitness for a rare allele at such a locus (with respect to the change in expression that it induces) is given by:

$$\frac{\partial W_M}{\partial X} = \frac{1}{2}\frac{\partial U}{\partial X} + \frac{1}{2}\frac{\partial V}{\partial X}. \tag{2.5}$$

Equation (2.5) differs from equation (2.2) because a rare allele at the modifier locus changes X in the current offspring, whether or not the offspring inherits the allele from its heterozygous mother. Thus, the effect on the fitness of the current offspring ($\partial U/\partial X$) is given only half the weight of an equivalent change in expression caused by mutation of a madumnal allele. This relationship would not hold for a *cis*-acting modifier expressed in mothers that was tightly linked to the primary locus. In this case, a rare allele at the modifier locus would only alter expression in one of the two alleles at the primary locus, and would (almost) always be transmitted along with that modified allele. Therefore, the fitness effect of such a modifier would be described by equation (2.2) rather than equation (2.5). That is, the *cis*/*trans* distinction is formally equivalent to the madumnal/maternal distinction.

By a similar argument, the change in paternal fitness for a rare allele at a *trans*-acting modifier locus expressed in fathers would be:

$$\frac{\partial W_P}{\partial X} = \frac{1}{2}\frac{\partial U}{\partial X} + \frac{k}{2}\frac{\partial V}{\partial X}. \tag{2.6}$$

The use of differential calculus in equations (2.5) and (2.6) assumes that erasures of epigenetic marks can be partial, allowing small changes in expression (not just all-or-none changes).

Both demand-enhancing and demand-inhibiting loci can be considered using equations (2.2–2.6). However, it will be useful to have a simple way of distinguishing the two kinds of loci. Therefore, in the remainder of this

paper, x_m, x_p and X will refer to levels of expression of demand enhancers, whereas y_m, y_p and Y will refer to levels of expression of demand suppressors. In the case of a demand enhancer, a rare allele causing a marginal increase in expression will result in a fitness benefit to the current offspring at a cost to the mother's residual reproductive value ($\partial U/\partial X > 0$, $\partial V/\partial X < 0$). Conversely, at a demand-suppressing locus, increased expression increases the mother's residual reproductive value at a cost to the offspring's fitness ($\partial U/\partial Y < 0$, $\partial V/\partial Y > 0$).

3. DEMAND ENHANCERS

Two simple relations follow from equations (2.2), (2.3) and (2.5).

$$\frac{\partial W_m}{\partial X} - \frac{\partial W_p}{\partial X} = \left(\frac{1}{2} - \frac{k}{2}\right)\frac{\partial V}{\partial X}, \tag{3.1a}$$

$$\frac{\partial W_m}{\partial X} - \frac{\partial W_M}{\partial X} = \frac{1}{2}\frac{\partial U}{\partial X}. \tag{3.1b}$$

From equations (2.4), (3.1*a*) and (3.1*b*), the following relations hold at demand-enhancing loci:

$$\frac{\partial W_p}{\partial X} > \frac{\partial W_{mp}}{\partial X} > \frac{\partial W_m}{\partial X} > \frac{\partial W_M}{\partial X}. \tag{3.2}$$

If each fitness function W_ξ has a single mode at $\hat{X}_\xi$ (where ξ represents any of M, P, m, mp or p and $\hat{X}_\xi > 0$), then (3.2) implies that

$$\hat{X}_p > \hat{X}_{mp} > \hat{X}_m > \hat{X}_M. \tag{3.3}$$

The location of $\hat{X}_P$, the optimal level of demand enhancer for paternal modifiers, always satisfies $\hat{X}_p \geqslant \hat{X}_P > \hat{X}_M$ when $0 \leqslant k < 1$, but its location with respect to $\hat{X}_m$ and $\hat{X}_{mp}$ depends on the value of k, such that

$$\begin{aligned} &\hat{X}_p \geqslant \hat{X}_P > \hat{X}_{mp} \text{ when } 0 \leqslant k < 1/3, \\ &\hat{X}_{mp} > \hat{X}_P > \hat{X}_m \text{ when } 1/3 < k < 1/2, \\ &\hat{X}_m > \hat{X}_P > \hat{X}_M \text{ when } 1/2 < k < 1. \end{aligned} \tag{3.4}$$

The critical values of $k = 1/3$ and $k = 1/2$ are evident as the crossover points in fig. 1*a* of Burt & Trivers (1998). If $X < \hat{X}_\xi$, novel alleles that increase X will be favoured at loci of type ξ. Conversely, if $X > \hat{X}_\xi$, alleles that decrease X will be favoured.

Suppose that a demand-enhancing locus were initially unimprinted, with an overall level of expression at the unimprinted optimum, $\hat{X}_{mp}$. Given these initial conditions, madumnal alleles that reduce X would be favoured, whereas padumnal alleles that increase X would be favoured. The resulting evolutionary dynamic, in which increases in padumnal expression are matched by decreases in madumnal expression, is predicted to runaway to complete silencing of madumnal alleles, $\hat{x}_m = 0$, with padumnal alleles producing their favoured amount $\hat{x}_p = \hat{X}_p$ (Haig 1996, 1997; Mochizuki *et al.* 1996; Wilkins & Haig 2001). The only evolutionarily stable state at an imprinted demand-enhancing locus is one in which the madumnal allele is silent. This conclusion follows directly from equation (3.1*a*): $\partial W_p/\partial X > \partial W_m/\partial X$ because $\partial V/\partial X < 0$.

The conclusion that $\hat{x}_m = 0$ and $\hat{x}_p = \hat{X}_p$ denote an evolutionarily stable state is based on the premise that natural selection acts solely on alleles at the primary locus. Is such a state also stable to selection acting on alleles at modifier loci? Maternal, paternal, madumnal and unimprinted modifiers would all favour reduced expression from the padumnal allele ($x_p < \hat{X}_p$), but none would favour reactivation of the silent madumnal allele. Thus, madumnal silencing at demand-enhancing loci is inherently stable, once established, although there is potential for ongoing conflict over the level of expression of the active padumnal allele.

Madumnal silencing could be achieved directly, by selection for DNA sequences that acquire epigenetic marks on transmission through female germ lines that result in inactivation of a sense promoter, or indirectly, by selection for DNA sequences that acquire epigenetic marks on transmission through male germ lines that result in inactivation of an antisense promoter. We will refer to these alternatives as the MS (madumnally silenced sense) and PA (padumnally silenced antisense) mechanisms, respectively.

In the analysis by Reik & Walter (2001*b*), there are 10 madumnally silent loci. Under the kinship theory of imprinting, these are candidate demand enhancers, although, in most cases, this has yet to be demonstrated. All 10 of the madumnally silent loci employ the MS mechanism and none uses the PA mechanism. A number of interrelated factors may explain the absence of PA loci. First, the MS mechanism may be easier to evolve because it is inherently simpler and because the active demethylation of the padumnal genome after fertilization obstructs the origin of the PA mechanism. Second, the MS mechanism may be selectively favoured over the PA mechanism because it does not involve antisense transcription and is thus less costly. Third, the MS mechanism, once established, would be robust to selection acting on maternal and paternal modifiers, or unimprinted modifiers of offspring. The PA mechanism would be vulnerable to maternal (and perhaps paternal) modifiers that activate the padumnal antisense promoter, inducing expression of padumnal antisense transcripts and suppressing padumnal sense transcripts. (If this suppression were total, neither allele at the primary locus would express sense transcripts and the locus would not be included in compilations of imprinted loci.)

4. DEMAND INHIBITORS

The equations (3.2) and (3.3) are reversed at demand-inhibiting loci:

$$\frac{\partial W_p}{\partial Y} < \frac{\partial W_{mp}}{\partial Y} < \frac{\partial W_m}{\partial Y} < \frac{\partial W_M}{\partial Y}, \tag{4.1}$$

$$\hat{Y}_p < \hat{Y}_{mp} < \hat{Y}_m < \hat{Y}_M. \tag{4.2}$$

At such loci, $\partial V/\partial Y > 0$. Therefore, $\partial W_p/\partial Y < \partial W_m/\partial Y$ and the only evolutionarily stable imprinted state is one in which the padumnal allele is silent (Wilkins & Haig 2001). If expression levels were determined solely by natural selection on alleles at the primary locus, the ESS would have the form $\hat{y}_m = \hat{Y}_m$, $\hat{y}_p = 0$. If overall expression of a demand inhibitor were $\hat{Y}_m$, madumnal modifiers would

favour the status quo, but unimprinted and padumnal modifiers would favour reduced expression of the madumnal allele ($y_m < \hat{Y}_m$), whereas maternal modifiers would favour increased Y, either by causing reactivation of the silent padumnal allele at the primary locus ($y_p > 0$) or by upregulation of the active madumnal allele ($y_m > \hat{Y}_m$).

The paternal optimum is always bounded by the padumnal and maternal optima ($\hat{Y}_p \leqslant \hat{Y}_P < \hat{Y}_M$), but its location relative to the madumnal and unimprinted optima depends on k (as in equation (3.4) above). If $k < 1/2$, then $\hat{Y}_P < \hat{Y}_m$ and paternal modifiers would favour reduced Y. However, if $k > 1/2$, then $\hat{Y}_P > \hat{Y}_m$ and paternal modifiers would favour an increase in Y, either by increasing expression from the madumnal allele at the primary locus, or by reactivating the silent padumnal allele. Thus, in the case of a demand inhibitor, not only is there potential for ongoing conflicts over the level of expression of the active madumnal allele, but maternal and paternal modifiers may also be selected to reverse padumnal silencing.

Padumnal silencing at a demand-inhibiting locus could be achieved directly, by epigenetic modification of a sense promoter in male germ lines, or indirectly, by epigenetic modification of an antisense promoter in female germ lines. We will refer to these alternative mechanisms as PS (padumnally silenced sense) and MA (madumnally silenced antisense). Reik & Walter (2001*a*,*b*) identify seven padumnally silent loci. As in the case of the madumnally silent loci, the function is not known for all of the gene products, but the kinship theory identifies them as candidate demand inhibitors. Of these seven padumnally silent loci, two employ the PS mechanism and five the MA mechanism. The PS mechanism may be difficult to evolve because of the active demethylation of the padumnal genome that occurs after fertilization. Moreover, long-term natural selection may favour the MA mechanism because it is evolutionarily robust to the action of parental modifiers. That is, at an MA locus, there is no incentive for maternal modifiers to activate the madumnal antisense promoter, whereas at PS loci there may be an incentive for maternal and/or paternal modifiers to activate padumnal sense promoters. If the padumnal sense promoter were reactivated, then a PS locus would be removed from the catalogue of imprinted genes.

To summarize §§ 3 and 4, demethylation of the padumnal genome immediately after fertilization provides a plausible constraint on the evolution of the PA and PS imprinting mechanisms and may thus help to explain why most imprinted loci employ either the MS or MA mechanism. This cannot be an absolute constraint, however, because Reik & Walter (2001*b*) identified two loci, *H19* and *Rasgrf1*, which use the PS mechanism.

Maternal modifiers may benefit from reactivation of silent padumnal promoters at loci encoding demand inhibitors. Could such reactivation be the adaptive reason for the *origin* of padumnal demethylation? The case for a positive answer would be strengthened if it were shown that imprinting evolved before demethylation, whereas such an answer could be rejected if demethylation evolved before imprinting. There is little evidence on this issue beyond the observation that, unlike the situation in mammals, genome-wide demethylation, followed by remethylation, does not occur in zebrafish (Macleod *et al.* 1999).

5. LOSS OF IMPRINTING

Our analysis identifies an asymmetry with respect to the long-term evolutionary stability of imprinting at madumnally and padumnally silent loci. The optimal level of padumnal expression of a demand enhancer at a madumnally silent locus ($\hat{X}_p$) is greater than the optimal levels for all of the potential modifiers considered in our analysis (see equation (3.3) above). Therefore, none of these potential modifiers would benefit from activating the silent madumnal allele if padumnal expression were $\hat{X}_p$. By contrast, the optimal level of madumnal expression of a demand inhibitor at a padumnally silent locus ($\hat{Y}_m$) is less than the optimal level for maternal modifiers ($\hat{Y}_M$) and less than the optimal level for paternal modifiers ($\hat{Y}_P$) if $k > 1/2$. Therefore, modifiers expressed in parents may be selected to activate silent madumnal alleles, with consequent loss of imprinting. The same does not apply to potential modifiers expressed in offspring (madumnal, padumnal or unimprinted) because activation of the madumnal allele would reduce their fitness (see equation (4.2) above).

In the above model, natural selection on parental modifiers favours loss of imprinting because this increases the production of a demand inhibitor. However, if imprinting is lost, natural selection on alleles at the inhibitor locus will favour reduced expression because the imprinted madumnal optimum ($\hat{Y}_m$) is greater than the unimprinted optimum ($\hat{Y}_{mp}$). In the short term, parents may benefit from the suppression of imprinting of demand inhibitors, but in the longer term this may result in increased demands by offspring. Natural selection lacks foresight.

The insulin-like growth factor 2 receptor gene (*IGF2R*) is padumnally silent in marsupials, rodents and artiodactyls, but not in monotremes or euarchontans (tree-shrews, flying lemurs and primates). By contrast, the insulin-like growth factor 2 gene (*IGF2*) is madumnally silent in marsupials, rodents, artiodactyls and primates, but not in monotremes (Killian *et al.* 2001*a*,*b*). This phylogenetic distribution is most parsimoniously explained by the origin of imprinting at both loci in an ancestral therian, with subsequent loss of imprinting at *IGF2R*, but not *IGF2*, in the euarchontan lineage. This pattern is consistent with the prediction that parental modifiers will favour loss of imprinting at padumnally silent loci but not at madumnally silent loci, but a single example is hardly strong evidence for the hypothesis. One would want several similar examples, and an absence of counter-examples, before feeling confident that an asymmetry existed in the evolutionary stability of imprinting at the two classes of loci.

6. WHO SUPPRESSES IMPRINTING?

§ 5 argues that the loss of imprinting at demand-inhibiting loci could be explained by the evolution of either paternal or maternal modifiers. Establishment of padumnal methylation patterns in the paternal germ line is likely to result from an interaction of *cis*-acting elements at the primary locus and *trans*-acting factors at modifier loci. The expected expression level at an unimprinted demand-inhibiting locus is $\hat{Y}_{mp}$. At such a locus, silencing of the padumnal promoter in the paternal germ line is always favoured by *cis*-acting elements, but is favoured by *trans*-acting factors only when $k < 1/3$. Therefore, the PS

mechanism of padumnal silencing at demand-inhibiting loci may be easier to evolve in highly polyandrous species because selection on *cis*-acting elements is not opposed by selection on *trans*-acting modifiers.

Conversely, the expression level at an imprinted demand-inhibiting locus is $\hat{Y}_m$. At such a locus, silencing of the padumnal promoter in the paternal germ line is always favoured by *cis*-acting elements, but is favoured by *trans*-acting factors only when $k < 1/2$. Thus, for $1/3 < k < 1/2$, natural selection on paternal modifiers may oppose the origin of imprinting at new loci but favour the maintenance of established imprints.

The maintenance of parent-specific methylation patterns in eight-cell mouse embryos and subsequent imprinted expression at these loci have recently been shown to depend on the genotype of an embryo's mother (Howell *et al.* 2001). This means that the maintenance of imprinting at this cell cycle depends on the products of maternal transcripts and increases the plausibility of maternal modifiers that act during the early stages of development to reactivate silent padumnal alleles.

7. LOSS OF IMPRINTING AND THE COST OF MUTATIONS

An alternative explanation for losses of imprinting has invoked the cost of functional hemizygosity at imprinted loci. That is, an imprinted locus will have a greater exposure to deleterious recessive mutations than an unimprinted locus, because one of its two alleles is transcriptionally silent (Sapienza 1989; Mochizuki *et al.* 1996). Spencer & Williams (1997) have shown that this selective force is very weak—of the same order as the mutation rate—and would usually be insignificant when there is positive selection for imprinting. However, their models consider germ-line mutations only. Fitness costs associated with somatic mutations (e.g. increased predisposition to cancer) are likely to provide a stronger selective force for biallelic expression, but this has yet to be formally modelled.

In its simplest form, the cost-of-mutation hypothesis does not predict an asymmetry between losses of imprinting at madumnally and padumnally silent loci. However, the existence of such an asymmetry could be reconciled with the hypothesis by invoking asymmetric costs of mutational inactivation at the two kinds of loci. For example, one might argue that somatic inactivation of the madumnal allele of an imprinted growth inhibitor, such as *IGF2R*, would predispose mutant cells to cancer, whereas somatic inactivation of the padumnal allele of an imprinted growth enhancer, such as *IGF2*, might have small or no consequences.

8. DISCUSSION

Genomic imprinting is often described as a manifestation of conflict between parents, played out within the genomes of their offspring. Such descriptions have probably been popular for two reasons. First, a model of conflict between the parents does capture certain critical attributes of the evolutionary forces underlying imprinting. Many systems of interest involve a greater maternal than paternal investment in offspring and the possibility of multiple paternity. Thus, similar forces act at the level of parents and at the level of alleles present in offspring that are inherited from those parents. Second, there exists a long tradition of work in behavioural ecology on the conflict between parents over the provision of care for offspring. This literature is more familiar to most biologists than the more recent work on intragenomic conflict, and is more easily reconciled with our everyday experience. The parental-conflict analogy therefore brings genomic imprinting into line with familiar concepts.

While the parental-conflict view of genomic imprinting may be adequate for some simple heuristic analyses, some models will require a more accurate accounting of evolutionary costs and benefits. Specifically, for some analyses it is important to distinguish the interests of an allele expressed in a parent from those of an allele derived from that parent, but expressed in offspring (Haig 1992; Burt & Trivers 1998). Our scenario for the loss of imprinting at a demand-inhibiting locus provides an example in which this distinction is crucial: maternal and paternal modifiers will sometimes favour demethylation of a padumnal sense promoter, but madumnal and padumnal modifiers will favour the status quo.

We have argued that madumnal silencing of demand enhancers is likely to be evolutionarily more robust than padumnal silencing of demand inhibitors. This asymmetry arises because $\hat{X}_p$, the padumnal optimum for an imprinted demand enhancer, is always greater than $\hat{X}_M$ and $\hat{X}_P$, the parental optima. Therefore, *trans*-acting modifiers expressed in parents, either mothers or fathers, will not benefit from the reactivation of silent madumnal alleles. By contrast, $\hat{Y}_m$, the madumnal optimum (the madumnal optimum for an imprinted demand inhibitor) for an imprinted demand inhibitor is always less than $\hat{Y}_M$ (the maternal optimum) and sometimes less than $\hat{Y}_P$ (the paternal optimum). Therefore, parental modifiers may benefit, at least in the short term, from reactivation of the silent padumnal allele.

In the discussion of their model, Burt & Trivers (1998) suggest that 'imprinting may be more stable over evolutionary time in the paternal germ line than in the maternal germ line, and that paternally imprinted genes should therefore come to outnumber maternally imprinted genes' (p. 2396). It is of interest to consider why our analysis has generated the opposite prediction. The prediction of Burt & Trivers follows in part from an assumption that 'maternal genes have so much more control than other genes over maternal investment' (p. 2396) and therefore that the paternal and padumnal interests will often be allied against the status quo. Our model assumes that silencing can be effected by *trans*-acting maternal factors, but that the expression level of an unsilenced allele is under the control of *cis*-acting elements; that is, under the control of the imprinted gene itself. The instability of padumnal silencing in our model follows from our assumption that a padumnally silent locus will be expressed at the madumnal optimum rather than the maternal one. The predictions made here are more consistent with the pattern observed by Reik & Walter (2001*a*,*b*), indicating that our assumptions may be more relevant to the evolution of imprinted gene expression. That is, the asymmetry in mechanisms of imprinting indicates that the expression level of an unmodified allele is

primarily the result of natural selection operating on *cis*-acting elements. The set of strategies available to parentally expressed *trans*-acting modifiers may be limited to allele-specific reduction of gene expression through epigenetic modification.

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