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Genomic imprinting of two antagonistic loci

Jon F. Wilkins* **and David Haig**

Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA

We present a model that considers the coevolution of genomic imprinting at a growth factor locus and an antagonistic growth suppressor locus. With respect to the two loci considered independently, our model makes the familiar predictions that an imprinted growth factor locus will only be expressed from the paternally derived allele and an imprinted growth suppressor locus only from the maternally derived allele. In addition, our coevolutionary model allows us to make predictions regarding the sequence of evolutionary events necessary for generating such a system. We conclude that imprinting at the growth factor locus preceded the evolution of growth suppressor function at the second locus, which in turn preceded imprinting at that locus. We then discuss the consistency of these predictions with currently available comparative data on the *insulin-like growth factor 2–insulin-like growth factor 2 receptor* system of mammals.

Keywords: genomic imprinting; *Igf2*; growth factor; growth suppressor; antagonistic coevolution; evolutionary stability

1. INTRODUCTION

Genomic imprinting refers to the phenomenon whereby some genes are expressed differently depending on whether they are inherited via an egg or a sperm. The kinship or parental conflict theory of imprinting (Trivers & Burt 1999; Haig 2000) proposes that imprinting evolves when the quantitative level of gene expression in an individual has fitness consequences for other individuals to whom the first has different degrees of matrilineal and patrilineal relatedness. When a gene is paternally derived, natural selection on expression levels favours alleles that increase patrilineal inclusive fitness, whereas when the gene is maternally derived, natural selection favours alleles that increase matrilineal inclusive fitness (Haig 1997, 2000). In the remainder of this paper we will follow the convention of using the adjectives madumnal and padumnal for denoting maternally derived and paternally derived alleles in offspring in contrast to the adjectives maternal and paternal, which are used to denote alleles in mothers and fathers (see Haig 1996).

Strong selection for differential gene expression can occur when offspring have an active role in acquiring resources from their mothers. In standard life-history theory, an offspring's optimal level of maternal investment is that which maximizes its inclusive fitness. The location of this optimum is governed by a trade-off between benefits to the offspring's personal fitness from increased investment and costs to sibs who receive less as a result, where these costs are discounted by their average coefficient of relatedness to the offspring (Trivers 1974). The kinship theory recognizes that the coefficient of patrilineal relatedness for these sibs will commonly be less than their coefficient of matrilineal relatedness due to the possibility of multiple paternity of a female's offspring. That is, a set of offspring who must compete for limited maternal resources are on average more closely related through their mother than through their father(s). Padumnal alleles therefore place greater weight on the benefit to the self in calculations of this trade-off, whereas madumnal alleles place greater weight on the cost to sibs.

The kinship theory predicts that the padumnal genome will favour higher expression than the madumnal genome at loci where increased expression acquires more maternal investment for an individual at a cost to its sibs. If the locus is unimprinted, the two alleles are constrained to exhibit the same expression level, and the evolutionarily stable level will occur somewhere between the two allelic optima. However, if the expression levels of the two alleles are able to evolve independently, the only evolutionarily stable expression pattern is one in which the madumnal allele is silent and the padumnal allele produces its preferred amount of the gene product (Haig 1997). Conversely, padumnal silence is predicted at imprinted loci where increased expression provides a benefit to sibs by reducing the individual's demands on its mother.

The paradigmatic examples of oppositely imprinted genes are the *insulin-like growth factor 2* (*Igf2*) and *insulin-like growth factor 2 receptor* (*Igf2r*) loci of mice (Haig & Graham 1991). In mice, *Igf2* is only expressed from the padumnal allele (DeChiara *et al.* 1991), whereas *Igf2r* is only expressed from the madumnal allele (Barlow *et al.* 1991). Inactivation of the padumnal allele of *Igf2* results in mice that are 60% of normal birth weight, whereas inactivation of the madumnal allele of *Igf2r* results in mice that are 140% of normal birth weight (Ludwig *et al.* 1996). The principal growth-related effect of *Igf2r* expression is the removal and degradation of the *Igf2* gene product (for a review, see O'Dell & Day 1998).

Below, we present a model of the evolution of genomic imprinting in a system of two loci with opposing effects on fetal growth. Our aim is to delineate plausible evolutionary scenarios for the coevolution of imprinting at antagonistic loci. In the context of the *Igf2–Igf2r* system, these scenarios can then be tested against comparative data (as it becomes available) from species in which one or both loci are unimprinted.

*Author for correspondence (jfwilkin@fas.harvard.edu).

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2. ASSUMPTIONS OF THE MODEL: STABILITY

Our model considers two loci, the first of which encodes a growth factor and the second a growth suppressor that acts by eliminating the product of the first locus. X (an individual's total production of growth factor) is the sum of x_m (production by the madumnal allele at the first locus) and x_p (production by the padumnal allele at that locus). Similarly, Y (the total production of the growth suppressor) is the sum of y_m (madumnal production) and y_p (padumnal production).

$$X = x_m + x_p, \tag{1}$$

$$Y = y_m + y_p, \tag{2}$$

and

$$x_m,\ x_p, y_m, y_p \geqslant 0. \tag{3}$$

The pattern of expression at the two loci in a given individual can be summarized by a four-element vector that is denoted by square brackets, i.e. $[x_m,\ x_p, y_m, y_p]$. This expression pattern is a property of an individual and should be distinguished from the expression strategies of alleles at the two loci. These will be represented by two-element vectors that are denoted by curly brackets, i.e. $\{x_m,\ x_p\}$ and $\{y_m, y_p\}$. Whereas x_m and x_p represent the (possibly different) expression levels of a single allele when it is maternally and paternally derived in a strategy vector, in a pattern vector x_m and x_p represent the expression levels of an individual's madumnal and padumnal gene copies, which may or may not represent different copies of the same allele.

An evolutionarily stable strategy (ESS) at the growth factor locus is defined as a strategy $\{\hat{x}_m,\ \hat{x}_p\}$ such that a population in which $\{\hat{x}_m,\ \hat{x}_p\}$ is adopted by most alleles is resistant to invasion by any rare alternative strategy $\{x_m,\ x_p\} \neq \{\hat{x}_m,\ \hat{x}_p\}$. Similarly, an ESS at the growth suppressor locus is defined as a strategy $\{\hat{y}_m, \hat{y}_p\}$ such that a population in which $\{\hat{y}_m, \hat{y}_p\}$ is adopted by most alleles is resistant to invasion by any rare alternative strategy $\{y_m, y_p\} \neq \{\hat{y}_m, \hat{y}_p\}$. The expression pattern of an individual who is homozygous for ESS alleles at both loci, i.e. $[\hat{x}_m,\ \hat{x}_p, \hat{y}_m, \hat{y}_p]$, will be called an evolutionarily stable pattern of expression (ESP) because a population in which this is the dominant pattern of expression is resistant to invasion by any rare allele that causes $[x_m,\ x_p, y_m, y_p] \neq [\hat{x}_m,\ \hat{x}_p, \hat{y}_m, \hat{y}_p]$.

An allele's strategy is only evolutionarily stable with respect to a specific set of alternatives. In this paper, we will consider two different types of strategy set. If a locus is 'imprinted', the set of possible strategies at the locus includes all pairs of non-negative madumnal and padumnal expression levels. If a locus is 'unimprinted', we only consider the subset of strategies that satisfies the additional constraint that the madumnal and padumnal expression levels are equal (i.e. $x_m = x_p = x_{mp}$ and/or $y_m = y_p = y_{mp}$). Thus, an ESS at an unimprinted locus may cease to be evolutionarily stable if the strategy set is expanded to include imprinted alleles.

The inclusive fitness of a rare autosomal allele W will be considered to be an equally weighted average of the matrilineal inclusive fitness (W_m) of its carriers when the allele is maternally derived and the patrilineal inclusive fitness (W_p) of its carriers when the allele is paternally derived, where W_m and W_p are calculated using coefficients of matrilineal and patrilineal relatedness rather than coefficients of average relatedness (see Haig 1997, 2000):

$$W = (W_m + W_p)/2. \tag{4}$$

W_m and W_p are given equal weights 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.

Our analysis will focus on the local stability of expression patterns. For this purpose, we will assume that the change in an individual's expression pattern caused by substituting a novel allele for the established allele is sufficiently small that we can estimate the relative fitness of the novel allele adequately with the first non-zero term in the series expansion:

$$\Delta W = \frac{\partial W}{\partial \xi}\Delta\xi + 0.5\frac{\partial^2 W}{\partial \xi^2}(\Delta\xi)^2 + O[(\Delta\xi)^3], \tag{5}$$

where ΔW represents the change in fitness relative to the established allele and ξ is the element of the expression vector under consideration. In these terms, a vector represents an ESP if $\Delta W < 0$ for all alleles in the strategy set with $\xi \geqslant 0$.

There are two conditions under which a vector element ξ could be evolutionarily stable. First, ξ could occupy a local fitness maximum. In this case, either an increase or a decrease in expression level would result in a decrease in fitness. Second, ξ could equal zero, in which case decreases in expression level are impossible and evolutionary stability simply requires that any increase in expression decreases fitness. These alternative criteria are summarized below:

$$\frac{\partial W}{\partial \xi} = 0,\ \frac{\partial^2 W}{\partial \xi^2} < 0 \tag{6}$$

and

$$\xi = 0,\ \frac{\partial W}{\partial \xi} < 0. \tag{7}$$

A rare allele that differs from the established allele in its madumnal expression only ($\xi = x_m$ or y_m) is without effects when paternally derived, i.e. $\partial W_p/\partial x_m = \partial W_p/\partial y_m = 0$. In such cases, we only need consider effects on matrilineal inclusive fitness, W_m. Likewise, for a rare allele that differs from the established allele solely in its padumnal expression ($\xi = x_p$ or y_p), $\partial W_m/\partial x_p = \partial W_m/\partial y_p = 0$ and we need consider only effects on patrilineal inclusive fitness, W_p. In contrast, W_m and W_p must both be considered at an unimprinted locus ($\xi = x_{mp}$ or y_{mp}).

3. INCLUSIVE FITNESS EFFECTS

The model assumes that the fitness effects of an allelic substitution are limited to its impact on the personal fitness of the individual in which the allele is expressed and its impact on the mother's future reproduction. An

individual's functional level of growth factor G is jointly determined by X and Y, where G increases monotonically with X and decreases monotonically with Y (except that G is equal to zero and invariant with respect to Y in the special case where $X=0$). The cost of realizing a particular value of G is given by the function $C(X, Y)$, which represents all costs to an individual's personal fitness resulting from resources being committed to the synthesis, processing and secretion of the growth factor and suppressor, as well as any energy expended in the removal and degradation of the growth factor by the suppressor. C is assumed to be a monotonically increasing function of both X and Y. U, an individual's personal fitness before deducting C, is assumed to increase monotonically with G. We also assume that the increased demand placed on the mother by an increase in G reduces the maternal reserves available for provisioning future offspring. Therefore, we assume that the mother's residual reproductive value V is a decreasing function of G.

The local stability criteria in equations (6) and (7) depend on the effects of changes in the gene expression levels on the inclusive fitness of a rare allele. The following first derivatives will be useful in our analysis:

$$\frac{\partial W_{\mathrm{m}}}{\partial X}=\left[\frac{\partial U}{\partial G}+0.5\frac{\partial V}{\partial G}\right]\frac{\partial G}{\partial X}-\frac{\partial C}{\partial X}, \tag{8}$$

$$\frac{\partial W_{\mathrm{p}}}{\partial X}=\left[\frac{\partial U}{\partial G}+p\frac{\partial V}{\partial G}\right]\frac{\partial G}{\partial X}-\frac{\partial C}{\partial X}, \tag{9}$$

$$\frac{\partial W_{\mathrm{m}}}{\partial Y}=\left[\frac{\partial U}{\partial G}+0.5\frac{\partial V}{\partial G}\right]\frac{\partial G}{\partial Y}-\frac{\partial C}{\partial Y}, \tag{10}$$

and

$$\frac{\partial W_{\mathrm{p}}}{\partial Y}=\left[\frac{\partial U}{\partial G}+p\frac{\partial V}{\partial G}\right]\frac{\partial G}{\partial Y}-\frac{\partial C}{\partial Y}. \tag{11}$$

In these equations, $\partial V/\partial G$ is multiplied by either p or 0.5, thereby indicating the expected fraction of the mother's future offspring that share a rare allele present in the current offspring. On average, half of these other offspring will share a rare madumnal allele, whereas some fraction p will share a rare padumnal allele. The value $2p$ can be thought of as the fraction of the mother's residual reproductive value that she shares with the father of the offspring possessing a rare padumnal allele (Lessells & Parker 1999). The value of p is a function of the mating system, which is assumed to be independent of X and Y.

At unimprinted loci, effects on both W_{m} and W_{p} must be considered and the relevant first derivatives are

$$\frac{\partial W}{\partial X}=0.5\left[\frac{\partial W_{\mathrm{m}}}{\partial X}+\frac{\partial W_{\mathrm{p}}}{\partial X}\right] \tag{12}$$

and

$$\frac{\partial W}{\partial Y}=0.5\left[\frac{\partial W_{\mathrm{m}}}{\partial Y}+\frac{\partial W_{\mathrm{p}}}{\partial Y}\right]. \tag{13}$$

Our model will assume that $0\leqslant p<0.5$, that is we assume that there is some probability that some of a mother's residual reproductive value is shared with male(s) other than the father of her current offspring. Subtraction of equation (8) from equation (9) gives

$$\frac{\partial W_{\mathrm{p}}}{\partial X}-\frac{\partial W_{\mathrm{m}}}{\partial X}=\left(p-0.5\right)\frac{\partial V}{\partial G}\frac{\partial G}{\partial X}. \tag{14}$$

The term on the right-hand side of equation (14) is always positive because $p<0.5$, $\partial V/\partial G<0$ and $\partial G/\partial X>0$. This implies

$$\frac{\partial W_{\mathrm{p}}}{\partial X}>\frac{\partial W}{\partial X}>\frac{\partial W_{\mathrm{m}}}{\partial X}. \tag{15}$$

This is to say that the marginal effect of an increase in X on the patrilineal inclusive fitness of padumnal alleles is always greater than the marginal effect of the same change on the matrilineal inclusive fitness of madumnal alleles. The marginal effect of the change on average inclusive fitness is of course intermediate between these values.

Similarly, subtraction of equation (10) from equation (11) yields

$$\frac{\partial W_{\mathrm{p}}}{\partial Y}-\frac{\partial W_{\mathrm{m}}}{\partial Y}=\left(p-0.5\right)\frac{\partial V}{\partial G}\frac{\partial G}{\partial Y}. \tag{16}$$

In this case, the term on the right-hand side of equation (16) will have the same sign as $\partial G/\partial Y$ because $p<0.5$ and $\partial V/\partial G<0$. By assumption of the model, $\partial G/\partial Y<0$ when $X>0$ and $\partial G/\partial Y=0$ when $X=0$. Therefore, if $X>0$

$$\frac{\partial W_{\mathrm{m}}}{\partial Y}>\frac{\partial W}{\partial Y}>\frac{\partial W_{\mathrm{p}}}{\partial Y}. \tag{17}$$

This is to say that the marginal effect of an increase in Y on the matrilineal inclusive fitness of madumnal alleles is always greater than the marginal effect of such a change on the patrilineal inclusive fitness of padumnal alleles.

However, if $X=0$, equations (10) and (16) give

$$\frac{\partial W_{\mathrm{m}}}{\partial Y}=\frac{\partial W}{\partial Y}=\frac{\partial W_{\mathrm{p}}}{\partial Y}=-\frac{\partial C}{\partial Y}<0, \tag{18}$$

i.e. decreases in Y always increase W_{m} and W_{p}. Therefore, natural selection will drive production of the growth suppressor to zero in the absence of the growth factor.

4. ESP CRITERIA (IMPRINTED LOCI)

At an ESP, all components of the pattern vector $[\hat{x}_{\mathrm{m}}, \hat{x}_{\mathrm{p}}, \hat{y}_{\mathrm{m}}, \hat{y}_{\mathrm{p}}]$ satisfy one of the stability criteria in equations (6) or (7). We will first consider the case in which both loci are imprinted, that is where all vector elements are free to vary independently and to take any non-negative value. At the growth factor locus, equation (15) implies $\partial W_{\mathrm{p}}/\partial x_{\mathrm{p}}>\partial W_{\mathrm{m}}/\partial x_{\mathrm{m}}$ because $\partial W_{\mathrm{p}}/\partial x_{\mathrm{p}}=\partial W_{\mathrm{p}}/\partial X$ and $\partial W_{\mathrm{m}}/\partial x_{\mathrm{m}}=\partial W_{\mathrm{m}}/\partial X$. Therefore, the requirement that $\partial W_{\mathrm{p}}/\partial x_{\mathrm{p}}=0$ at an ESP implies $\partial W_{\mathrm{m}}/\partial x_{\mathrm{m}}<0$. In other words, if the growth factor locus is imprinted, madumnal alleles must be silent ($\hat{x}_{\mathrm{m}}=0$).

Zero padumnal production of the growth factor ($\hat{x}_{\mathrm{p}}=0$) is predicted if

$$\frac{\partial W_{\mathrm{p}}}{\partial x_{\mathrm{p}}}=\frac{\partial W_{\mathrm{p}}}{\partial X}\leqslant 0 \text{ when } X=0. \tag{19}$$

If equation (19) is satisfied, equation (18) implies that there will also be zero production of the growth suppressor at the ESP. If equation (19) is not satisfied, the ESP will occur at a fitness maximum of padumnal expression ($\partial W_p/\partial x_p = 0$ and $\partial^2 W_p/\partial x_p^2 < 0$).

At the growth suppressor locus, equation (17) implies $\partial W_p/\partial y_p < \partial W_m/\partial y_m$ because $\partial W_p/\partial y_p = \partial W_p/\partial \Upsilon$ and $\partial W_m/\partial y_m = \partial W_m/\partial \Upsilon$. The ESP requirement that $\partial W_m/\partial y_m = 0$ implies $\partial W_p/\partial y_p < 0$. Therefore, if the growth suppressor locus is imprinted, padumnal alleles must be silent at an ESP ($\hat{y}_p = 0$).

Zero madumnal production of the growth suppressor ($\hat{y}_m = 0$) is predicted if

$$\frac{\partial W_m}{\partial y_m} = \frac{\partial W_m}{\partial \Upsilon} \leqslant 0 \text{ when } \Upsilon = 0. \tag{20}$$

Otherwise, an ESP will occur at a fitness maximum of madumnal expression ($\partial W_m/\partial y_m = 0$ and $\partial^2 W_m/\partial y_m^2 < 0$).

In summary, if the growth factor locus is imprinted, the only possible ESPs are ones in which the madumnal allele is silent. Conversely, if the growth suppressor locus is imprinted, the only possible ESPs are ones in which the padumnal allele is silent. If equation (19) is satisfied, the ESP will have the form [0, 0, 0, 0]. If equation (20) is satisfied but not equation (19), the ESP will have the form [0, $\hat{x}_p$, 0, 0]. If neither of equations (19) and (20) is satisfied, the ESP will have the form [0, $\hat{x}_p$, $\hat{y}_m$, 0].

5. CONSTRAINED ESPS (UNIMPRINTED LOCI)

We first consider the conditions for zero production at unimprinted loci. If the growth factor locus is unimprinted, zero production of the growth factor ($\hat{x}_{mp} = 0$) is predicted at an ESP if

$$\frac{\partial W}{\partial x_{mp}} = \frac{\partial W}{\partial X} \leqslant 0 \text{ when } X = 0. \tag{21}$$

It can be seen from equation (15) that equation (21) is satisfied if equation (19) is satisfied, but not vice versa. In other words, padumnal-specific expression of the growth factor can be favoured under some conditions for which zero production would be favoured if the locus were unimprinted. If equation (21) is satisfied, equation (18) implies that there will also be zero production of the growth suppressor at the ESP regardless of whether the growth suppressor is imprinted or unimprinted.

If the growth suppressor locus is unimprinted, zero production of the growth suppressor ($\hat{y}_{mp} = 0$) is predicted at an ESP if

$$\frac{\partial W}{\partial y_{mp}} = \frac{\partial W}{\partial \Upsilon} \leqslant 0 \text{ when } \Upsilon = 0. \tag{22}$$

Equations (17) and (18) imply that equation (22) is satisfied if equation (20) is satisfied, but not vice versa. In other words, madumnal-specific expression of the growth suppressor can be favoured under some conditions for which zero production would be favoured if the locus were unimprinted.

We now consider the case where the growth factor is unimprinted but the growth suppressor is imprinted. If equation (21) is not satisfied, the ESP for an unimprinted growth factor will occur when $\partial W/\partial x_{mp} = \partial W/\partial X = 0$. Substituting equations (8), (9) and $\partial W/\partial X = 0$ into equation (12) gives

$$\frac{\partial U}{\partial G} + \left(\frac{1+2p}{4}\right)\frac{\partial V}{\partial G} = \frac{\partial C}{\partial X}\Big/\frac{\partial G}{\partial X}. \tag{23}$$

Since both $\partial C/\partial X$ and $\partial G/\partial X$ are positive, the left-hand side of equation (23) must be positive at an ESP of this type. Substituting $\partial W_m/\partial \Upsilon = 0$ (the stability criterion for an imprinted growth suppressor) into equation (10) gives

$$\frac{\partial U}{\partial G} + 0.5\frac{\partial V}{\partial G} = \frac{\partial C}{\partial \Upsilon}\Big/\frac{\partial G}{\partial \Upsilon}. \tag{24}$$

The left-hand side of equation (24) must be negative because $\partial G/\partial \Upsilon$ is negative. Therefore, if neither of equations (19) and (21) is satisfied, an ESP must satisfy the joint conditions

$$\frac{\partial U}{\partial G} + 0.5\frac{\partial V}{\partial G} < 0 < \frac{\partial U}{\partial G} + \left(\frac{1+2p}{4}\right)\frac{\partial V}{\partial G}. \tag{25}$$

These conditions do not preclude an ESP of the form [$\hat{x}_{mp}$, $\hat{x}_{mp}$, $\hat{y}_m$, 0] because $\partial V/\partial G < 0$ and $p < 0.5$.

We will next consider the case where neither locus is imprinted. Equation (23) is the ESP condition at the growth factor locus. At the growth suppressor locus, substituting the right-hand side of equation (23) into equation (13) gives

$$\frac{\partial W}{\partial \Upsilon} = \left\{\frac{\partial C}{\partial X}\Big/\frac{\partial G}{\partial X}\right\}\frac{\partial G}{\partial \Upsilon} - \frac{\partial C}{\partial \Upsilon}. \tag{26}$$

The quantity within the brackets and $\partial C/\partial \Upsilon$ are always positive, whereas $\partial G/\partial \Upsilon$ is always negative or zero. Therefore, $\partial W/\partial \Upsilon$ must be negative and equation (22) is always satisfied. If equation (21) is satisfied both loci will be unexpressed, otherwise the ESP will have the form [$\hat{x}_{mp}$, $\hat{x}_{mp}$, 0, 0]. An ESP of the form [$\hat{x}_{mp}$, $\hat{x}_{mp}$, $\hat{y}_{mp}$, $\hat{y}_{mp}$] is specifically excluded.

Finally, we consider the case of an imprinted growth factor and unimprinted growth suppressor. If neither of equations (19) and (22) is satisfied, $\partial W/\partial \Upsilon = \partial W_p/\partial X = 0$ at an ESP. Substituting these values into equations (9) and (13) gives a joint condition analogous to equation (25), i.e.

$$\frac{\partial U}{\partial G} + \left(\frac{1+2p}{4}\right)\frac{\partial V}{\partial G} < 0 < \frac{\partial U}{\partial G} + p\frac{\partial V}{\partial G}. \tag{27}$$

The model does not preclude an ESP of the form [0, $\hat{x}_p$, $\hat{y}_{mp}$, $\hat{y}_{mp}$] because $\partial V/\partial G < 0$ and $p < 0.5$.

6. APPLICATION TO THE *IGF2–IGF2R* SYSTEM

The model presented above can be applied in understanding the evolution of the *Igf2–Igf2r* system. For this interpretation, X represents the expression level of the growth-enhancing factor *Igf2*, Υ the expression level of *Igf2r* and G the steady-state level of Igf2. As such, the model fails to capture at least one important aspect of the biology of Igf2r. This molecule's ancestral function is as the cation-independent mannose 6-phosphate receptor (CI-MPR), which facilitates endocytosis of molecules adorned with mannose 6-phosphate residues, targeting them to lysosomes. In marsupials and eutherian mammals,

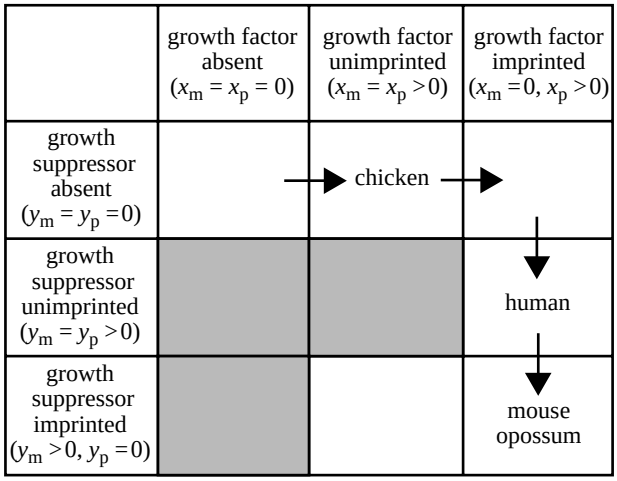

Figure 1. Summary of the possible evolutionarily stable patterns of expression for a system of two antagonistic loci of the *Igf2–Igf2r* type. The shaded cells are those combinations of characters that are precluded by the model. Species for which the expression pattern at these two loci has been well-established are also presented. Note that novel alleles that increase or decrease the quantitative expression level of an existing allele represent expression changes within a single box in this table. Transitions between orthogonally adjacent boxes arise from the introduction of a novel allele that either creates or eliminates a gene function or originates or eliminates imprinting at a locus. Transitions from the first column directly to the third or from the top to the bottom row are possible only in the case where a novel gene function evolves at a previously imprinted locus. Our proposed historical scenario for the evolution of imprinting in the *Igf2–Igf2r* system is represented by a three-step sequence beginning in the upper-middle box, moving across to the top-right corner and then down the right-hand column to the bottom right box, which represents the situation where both loci are imprinted.

CI-MPR has evolved a novel binding site for Igf2 and has thereby acquired the additional function of degrading Igf2. The CI-MPR does not bind Igf2 in monotremes or birds (Killian *et al.* 2000).

The model's prediction that there should be zero expression of an unimprinted growth suppressor in the absence of imprinting at the growth factor locus follows directly from the assumption that the only function of the growth suppressor is to eliminate the growth factor. However, it is clear that *CI-MPR* would have had non-zero expression prior to its acquisition of an Igf2-binding site, and that production of Igf2r/CI-MPR has fitness consequences that are independent of its effects on Igf2. The model can be brought into closer correspondence with reality by reinterpreting Υ as being a measure of the Igf2-degrading capacity of the CI-MPR. Thus, in the absence of the Igf2 binding site, $\Upsilon = 0$ regardless of the receptor's expression level. Once a binding site had evolved, Υ became positive and increased with increases in Igf2-binding affinity and with increases in the production of Igf2r/CI-MPR. The assumption that $\partial C/\partial \Upsilon > 0$ remains reasonable under this redefinition, even when changes in Υ are due to changes in binding affinity rather than changes in expression level because endocytosis and degradation of Igf2 carry metabolic costs.

Our analysis suggests a scenario for the evolution of imprinting of *Igf2* and *Igf2r* (figure 1). At first, *Igf2* was unimprinted and the CI-MPR neither bound nor degraded Igf2, i.e. $[\hat{x}_{mp}, \hat{x}_{mp}, 0, 0]$. This pattern of expression was destabilized by the origin of imprinting at *Igf2*, which favoured silencing of madumnal *Igf2* alleles and an increase in padumnal expression to the level that optimized W_p, i.e. $[0, \hat{x}_p, 0, 0]$. Increased levels of Igf2 created a selective force that favoured the acquisition of the Igf2-binding site by an unimprinted *Igf2r*, $[0, \hat{x}_p, \hat{y}_{mp}, \hat{y}_{mp}]$. Finally, the origin of imprinting at *Igf2r* favoured silencing of padumnal alleles and an increase in madumnal expression to the level that maximized W_m, i.e. $[0, \hat{x}_p, \hat{y}_m, 0]$. Thus, this scenario translates into a prediction that the evolution of imprinting at *Igf2* preceded the acquisition of an Igf2-binding site by CI-MPR (and its transmogrification into *Igf2r*), which in turn preceded the evolution of imprinting at *Igf2r*.

The above scenario makes three predictions about which combinations of characters will occur together when the *Igf2–Igf2r* system is studied in diverse taxa. First, madumnal silencing of *Igf2* will only occur in taxa in which enhanced growth of an offspring has negative fitness consequences for its mother's other offspring. Second, the Igf2-binding site of CI-MPR will exist only in taxa in which *Igf2* is (or has been) imprinted. Third, padumnal silencing of *Igf2r* will occur only in taxa in which CI-MPR binds Igf2.

These predictions are consistent with all data currently available, but the data are as yet inadequate for eliminating alternative scenarios. *Igf2* is known to be imprinted in various muroid rodents (DeChiara *et al.* 1991; Pedone *et al.* 1994; Vrana *et al.* 1998), sheep (Feil *et al.* 1998), humans (Ohlsson *et al.* 1993) and the opossum *Monodelphis domestica* (O'Neill *et al.* 2000). Imprinting of *Igf2* is believed to be restricted to mammals, although the only organism in which *Igf2* has actually been demonstrated to be unimprinted is the chicken (O'Neill *et al.* 2000). Thus, all species in which *Igf2* has been shown to be imprinted have extensive post-zygotic maternal care and the data are compatible with a single origin of *Igf2* imprinting in a common ancestor of eutherian mammals and marsupials (whether *Igf2* is imprinted in monotremes is currently unknown).

CI-MPR has an Igf2-binding site in eutherian mammals and marsupials (Dahms *et al.* 1993; Yandell *et al.* 1999; Killian *et al.* 2000), but not in monotremes (Killian *et al.* 2000), chickens or *Xenopus* (Clairmont & Czech 1989). Thus, all taxa in which CI-MPR is known to have an IGF2-binding site are also known to imprint *Igf2*, whereas no taxon is known to imprint *Igf2* but lack the binding site. These data are consistent with the prediction that *Igf2* imprinting preceded evolution of the binding site, but are also consistent with the reverse sequence.

Comparative data on *Igf2r* imprinting are limited. The *Igf2r* loci of mice (Barlow *et al.* 1991) and the opossum *Didelphis virginiana* are known to be imprinted (Killian *et al.* 2000), whereas the *Igf2r* locus of humans (Kalscheuer *et al.* 1993) and the *CI-MPR* locus of monotremes (Killian *et al.* 2000) are biallelically expressed. Thus, our historical scenario is not contradicted by any species that is known to have *CI-MPR* imprinting but lack the Igf2-binding site.

The absence of *Igf2r* imprinting in humans could be interpreted as evidence that the acquisition of the Igf2-binding site preceded two independent origins of *Igf2r* imprinting in mice and *Didelphis*, but unpublished data have suggested that *Igf2r* imprinting has been lost in primates (K. Killian, personal communication). Our model does not predict losses of imprinting, and any such loss would pose a theoretical challenge that would probably require the addition of costs of imprinting to the model.

We have predicted that imprinting of *Igf2* preceded both the evolution of the growth suppressor function and imprinting of *Igf2r*. This conclusion is predicated on the assumption that *CI-MPR* was not already imprinted for some other reason independent of its interaction with Igf2. Padumnal silence at a locus favours the acquisition of additional functions that reduce growth factor levels and enhance W_m (whether or not the growth factor loci are imprinted), because these new functions are expressed only when an allele is maternally derived (Haig 2000). Although the model precludes the acquisition of a growth-suppressing function at an unimprinted locus if the growth factor locus is not imprinted, this conclusion does not hold if the 'growth suppressor' were already padumnally silent. In other words, $[\hat{x}_{mp}, \hat{x}_{mp}, 0, 0]$ and $[\hat{x}_{mp}, \hat{x}_{mp}, \hat{y}_{mp}, 0]$ are possible ESPs, but $[\hat{x}_{mp}, \hat{x}_{mp}, \hat{y}_{mp}, \hat{y}_{mp}]$ is not. Therefore, alternative scenarios would be possible if *CI-MPR* were padumnally silent (for some independent reason) prior to acquiring its Igf2-binding site.

7. STABILITY AND METASTABILITY

The above discussion of evolutionary scenarios and alternative ESPs is based on an implicit assumption that some classes of mutations are more common than others. It would be evolutionarily meaningless to talk about 'ESPs' at unimprinted loci being replaced by ESPs at imprinted loci unless mutations that confer imprinting upon a previously unimprinted locus are much rarer than mutations that simply increase or decrease gene expression. Similarly, the inclusion of $[0, \hat{x}_p, 0, 0]$ as an intermediate stage in our scenario (*Igf2* imprinted and CI-MPR lacking an Igf2-binding site) assumes that mutations that change levels of gene expression are much more common than mutations that generate a novel function for a gene product (in this case, the capacity to bind and eliminate Igf2). Thus, $[0, \hat{x}_p, 0, 0]$ could be considered evolutionarily stable under the constraint that alleles at the second locus are unable to suppress the growth factor. Alternatively, one might call this a metastable state that is destabilized once a rare mutation (or set of mutations) that generates a growth-suppressing function occurs. Likewise, $[0, \hat{x}_p, \hat{y}_{mp}, \hat{y}_{mp}]$ is metastable under the constraint that the second locus is unimprinted, but becomes unstable once a rare mutation (or set of mutations) confers imprinting upon that locus.

Whether a given state is globally stable or merely metastable with respect to a rare function-creating mutation will depend on one or more of equations (19)–(22). Within the terms of our model, any unimprinted ESP with non-zero gene expression is always destabilized by the introduction of imprinted alleles. However, such an unimprinted ESP might be globally stable if imprinting carried an inherent fitness cost (such as an increased risk of functional hemizygosity for deleterious alleles).

8. PREVIOUS MODELS

At least three previous models have considered the evolution of imprinting at growth-enhancing and growth-suppressing loci.

Mochizuki *et al.* (1996) presented a model in which padumnal-only expression of a growth enhancer evolves when $p < 0.5$. Their Appendix C extended their model to the evolution of madumnal-only expression of a growth suppressor, but only under the assumption of a fixed positive level of growth factor production (F_m), which was chosen to allow evolution of imprinting at the suppressor locus but was not itself required to satisfy any criterion of evolutionary stability.

Spencer *et al.* (1998) presented a series of diallelic models in which one allele was unimprinted and the other was imprinted, but in which the levels of expression were fixed, given that an allele had non-zero expression. Their models were sufficiently general to apply to either a growth-enhancing or a growth-suppressing locus, but are not comparable with the model presented in this paper because the question of evolutionary stability against the introduction of novel mutations was not addressed (see Haig 1999).

Haig & Wilkins (2000) presented a model of scramble competition within litters in which imprinting at demand-enhancing and demand-reducing loci were able to coevolve. Their model predicted padumnal-only expression of the demand enhancer and madumnal-only (or zero) expression of the demand inhibitor. The model of Haig & Wilkins (2000) differed from the model presented in this paper in that it considered simultaneous rather than sequential production of offspring and did not consider transitions between imprinted and unimprinted states.

9. CONCLUSION

The model presented in this paper addresses the coevolution of genomic imprinting at two antagonistic loci, one encoding a growth factor and the other a growth suppressor, that functions through the degradation of the growth factor. We have shown that the growth factor locus, if imprinted, will be madumnally silent. Similarly, we have shown that an imprinted growth suppressor locus will be padumnally silent. Furthermore, we have presented an argument that the development of such a growth suppressor is not favoured until the growth factor locus has become imprinted. This has allowed us to hypothesize a historical scenario for the evolution of the *Igf2*–*Igf2r* system. We have attempted to make a number of specific predictions with respect to this system that we hope will be scrutinized against comparative data from diverse taxa as they become available.

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