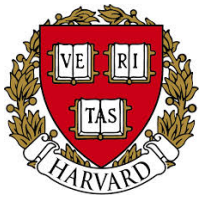

Master thesis

Can responsive genomic strategies alleviate genomic conflicts?

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Abstract

Genomic imprinting has been predicted to evolve when paternity is uncertain as an attempt to increase paternal line's fitness. Imprinting at one locus may destabilise other loci causing reciprocal imprinting at antagonistic loci. Using a model of a growth promoter and a growth inhibitor (such as $Igf2$-$Igf2r$) and assuming unresponsive genetic strategies, reciprocally imprinted antagonistic loci have been mathematically predicted. However it is well know that cellular sensing and physiological responses are crucial for an accurate development. Here I relax the assumption of unresponsive genes by introducing a maternal physiologically responsive strategy and study how genes' expression level may evolve. This is, making the expression at the inhibitor locus as a function of the promoter's total expression. Then, I restrict the analysis to linear functions to compare the effects of different slopes. While the evolution of the responsiveness strictly depends on asymmetric parental relationships (paternal uncertainty), I demonstrate that the maternal responsive strategy is able to reduce expression levels compared to unresponsive gene strategies. The strength of gene's expression reduction is dependent on the slope of the function; steeper slopes achieve lower levels of expression. These responsive strategies suggest a reallocation of reproductive efforts, from current to future offspring. All results are also shown by a graphical analysis of a particular case. This work is a first attempt to formally describe the evolution of gene expression and the evolution of genomic interactions by mathematical implementations of physiological responsive strategies.

1 Introduction

Gene imprinting might have important implications in biological evolution as it has great impact in individuals' fitness according to the allele expressed (Haig and Wilkins, 2000). It has been shown that imprinting patterns are essential during development, and that specific allelic expression shapes the phenotype of the offspring (Latham et al., 1994; Cowley et al., 2014). For instance, abnormal gene imprinting patterns have been associated with a wide range of embryonic and fetal abnormalities (Moore and Reik, 1996), alterations in the development and function of the placenta (Gutierrez-Marcos et al., 2012) and fetal death (Dória et al., 2010). A gene is considered to be imprinted when it changes its expression pattern depending on whether it is maternally or paternally inherited, thus an imprinted gene presents exclusively maternal or paternal allelic expression (Hurst et al., 1996; Lawson et al., 2013). It has been proposed that genetic, or genomic, imprinting is a solution of genetic (or genomic) conflicts between different genetic elements over the state of a trait that usually is related to individual's fitness (Haig and Westoby, 1989; Ross et al., 2010). Genomic conflicts occur when a self-promoting genetic element creates the context for the spread of another genetic element with opposite effect expressed in the same individual (Hurst et al., 1996).

One possible explanation for genetic conflicts to emerge are asymmetric kin relationships (Haig, 2004). Especially during parental conflicts, acting in the offspring's genome, selection forces are expected to be the strongest (Haig, 1997; Patten et al., 2014). Haig and Westoby (1989) proposed that genomic imprinting can evolve in systems where females can produce offspring conceived by more than one male during their life span and the offspring receives postfertilisation nutrients from one

of the parents. The latter condition implies that siblings compete for a limited pool of resources, usually supplied by the mother. On the other hand, if her offspring is fathered by different males, the probability of a paternal allele being shared by other offspring is reduced. Then, it could be in the interest of paternal alleles to demand more resources from the mother to increase the survival probability of the current offspring, by silencing maternally derived alleles of genes that enhance current offspring's individual fitness (Haig, 2004). However, imprinting at one locus could have differential fitness consequences by destabilising other loci expression. For instance, the mismatch between the paternal and maternal optimal level of demand imposes to the maternal alleles a trade off between current and future reproduction (Haig, 2004; Moore and Reik, 1996). Then, as a second selective step, the acquisition of a "repressor" to the self-promoting genetic element may be possible (Wilkins and Haig, 2001). Selection may favor the maternal expression of any inhibitor to the increased demand, either already present or to be acquired (Wilkins and Haig, 2001; Patten et al., 2014).

Flowering plants and therian mammals are two systems that meet the conditions exposed by Haig and Westoby (1989) for imprinting to evolve. In angiosperms imprinted loci are mostly expressed in the endosperm and in the embryo, over 300 imprinted genes have been reported in the endoderm. Similarly, imprinted genes in therians seem to coevolve with the placenta and the maternal-fetal interaction, over 150 imprinted genes have been reported in mice placenta (Gutierrez-Marcos et al., 2012). For both taxa, there is empirical evidence showing that genes controlling food supply are preferentially maternally expressed, while genes controlling the demand tend to be paternally expressed (Moore and Reik, 1996). This evidence is in accordance with the predictions of the kinship theory that genes controlling embryo growth, since it affects its fitness, are prone to be imprinted (Haig, 2004).

As described previously, the kinship theory also predicts imprinting at one locus as a response to previous imprinting events at other loci, thus the existence of reciprocally imprinted antagonistic genes (Wilkins and Haig, 2001). The best studied pair of antagonistic genes ($Igf2$-$Igf2r$) has been described in eutherians (placental mammals) (Patten et al., 2014). The *insulin-like growth factor 2* ($Igf2$) is a protein that promotes embryonic growth and it is paternally expressed. In contrast, the $Igf2$ *receptor* ($Igf2r$) is a membrane receptor for mannose 6-phosphate-labeled ligands targeted to lysosomes with an independent binding site for $Igf2$, therefore it inhibits embryonic growth; it is maternally expressed, except in Euarchonta (primates and relatives) which have biallelic expression (Killian et al., 2000; Haig, 2004). Other two maternally expressed genes have been suggested as possible antagonist of $Igf2$, the first one is $H19$ a noncoding but highly expressed RNA that may control the level of expression of $Igf2$ possibly by posttranscriptional interactions. The second one is the *cyclin-dependent kinase inhibitor 1c* (CDKN1C) which has been suggested to have effects on cell proliferation opposed to the ones of $Igf2$ (Haig, 2004). Thus supports the idea of conflicting parties rather than gene by gene conflicts where several loci are associated with a self-promoting element and some other loci suppressing their effect (Hurst et al., 1996).

Recently, another pair of reciprocally imprinted genes ($Dlk1$-$Grb10$), independent fo the $IGF2$ pathway, has been described in mice (Madon-Simon et al., 2014). The *growth factor receptor bound protein 10 gene* ($Grb10$) controls postnatal food supply by the mother and demand by the offspring, as well as determines fat-lean deposition during development. It is maternally expressed and functions as a growth inhibitor although allows fat deposition (Cowley et al., 2014). On the other hand, *delta-like 1* ($Dlk1$) is paternally expressed and acts upstream of $Grb10$ as its inhibitor, hence promoting offspring growth with higher lean proportion (Madon-Simon et al., 2014). The evolution of reciprocally imprinted genes is feasible under the kinship theory (Haig and Wilkins, 2000; Wilkins and Haig, 2001) and $Igf2$-$Igf2r$ and $Dlk1$-$Grb10$ are clear examples of antagonistic gene pairs that are consistent with the kinship theory, where the paternally expressed gene promotes offspring's fitness at the expense of the mother's (Wilkins and Haig, 2001; Haig, 2014).

All previous theoretical work on the evolution of reciprocally imprinted genes assumes that selective forces acting on maternal alleles are independent from the ones on paternal alleles (Patten et al., 2014). This assumption implies that the level of expression at one locus achieves its optimum level independently of the expression level at the other locus (Haig and Wilkins, 2000; Wilkins and Haig, 2001). Silencing one parental line allows the other to attain the level of expression that maximises its own benefits (Haig, 1997). However, if the paternal allele increases the expression of the growth

promoter to increase it's own fitness, it should be in the interest of, and may be optimal for, the maternal alleles to immediately (physiologically) increase the level of repression, as maternal fitness is threatened. Hence, the nature of these antagonistic loci could imply that the allele's expression level at one locus is adjustable to the total expression at the other locus as a physiological response, and before selection. This reactionary interaction has never been considered by previous work, as the genes are considered to be involved in an uninformed game. However the genetic interactions take place in each cell of the offspring, where molecular sensing and thresholds are the cues for accurate offspring development (Costa et al., 2012). Therefore, physiological conditional behaviours could have remarkable evolutionary relevance. The physiological adjustment of allelic expression suggests that alleles would tend to lower their expression level to reduce the physiological costs of production of their products. But, can imprinted genes bargain their expression level? Are there evolutionarily stable equilibria that minimise conflict costs? If one of the locus is able to condition its expression level to the total expression at the other locus, would it be in the interest of the latter to change its expression level?

The aim of this work is to evaluate whether the level of expression of reciprocally imprinted genes can be modified if one of the locus can physiologically condition its expression level to the expression level at the other locus; compared to unresponsive strategies that cannot condition its level of expression. I follow a theoretical approach taking the model by Wilkins and Haig (2001) and relaxing their assumption of independent selection. The physiological responsive strategy is modelled by making the expression level at one locus a functional response on the total expression at the other locus. I assume that the loci are already imprinted to study the evolutionary implications of this responsive strategy on the expression level. Then I evaluate if there exist solutions that can lower the cost of the system and obtain the equilibrium condition for the responsive strategy, as well as analyse the conditions to be met for a responsive strategy to evolve. Finally, I constuct a particular example to graphically compare the effects of different maternal responsive strategies.

2 Model

Based on an antagonistic system with a growth promoter and an inhibitor that acts by eliminating the first (as $Igf2$-$Igf2r$), Wilkins and Haig (2001) constructed a model to study the evolution of reciprocal imprinting. The authors conclude that silencing of one parental can be achieved, and evaluate the stability of the expressing allele. Nonetheless, their results are derived from the assumption of independent selection on the genetic level of expression at each locus. In this work I relax that assumption by introducing a responsive strategy on the maternally expressed growth inhibitor, which can condition its expression level to the total expression of the growth promoter. Wilkins and Haig's model considers the total production of growth factor (X) as the sum of the production by the maternally (x_m) and paternally (x_p) derived alleles. Similarly, the total production of growth inhibitor (Y) is the sum of the production by the parental alleles (y_m and y_p). Thus, by definition x_m, x_p, y_m, $y_p \geq 0$, and

$$X = x_m + x_p$$
$$Y = y_m + y_p$$

However, following an evolutionary logic, I consider that alleles would evolve responsive strategies only when loci are already imprinted. Hence, the level of expression at the growth promoter locus is $X = 0 + x_p$ (paternally expressed), while at the growth inhibitor is $Y = y_m + 0$ (maternally expressed), with $x_p, y_m > 0$; to keep notation simple let $x = x_p$ and $y = y_m$.

2.1 Strategies

2.1.1 Unresponsive strategy

Classically, it is assumed that genes are under independent selective pressure for the total level of expression at each locus (Wilkins and Haig, 2001; Patten et al., 2014), this means that for a given

value of X, Y can explore all possible values of its domain and vice versa, therefore:

$$X = x \tag{1a}$$
$$Y = y \tag{1b}$$

2.1.2 Maternal threat

To introduce the responsive strategy, I consider that the repressor locus is able to condition its total expression level. In the $Igf2$-$Igf2r$ system, the repressor role is taken by the maternally imprinted $Igf2r$. Notice that in the $Dlk1$-$Grb10$ system the repressor locus is the paternally imprinted gene $Dlk1$, however the mathematical derivation of results under a paternal threat would be analogous. For this analysis where $Igf2$-$Igf2r$ system is considered, the maternally expressed growth inhibitor ($Igf2r$, now y) is the one considered to respond to the total expression of the paternally expressed growth promoter ($Igf2$, now x). The resulting allelic expression is

$$X = x \tag{2a}$$
$$Y = y(x) \tag{2b}$$

where the Y locus is a mathematical generalisation of the non responsive strategy which allows Y to be a function of x istead of a fixed value. This is, the expression at the growth inhibitor locus is a function of the level of expression at the growth promoter locus. The growth inhibitor locus should increase the expression level in response to an increased expression of the growth promoter, but diminish its expression if the growth promoter diminishes expression, this means that $y(x)$ is an increasing function, then $y'(x) > 0$.

As the aim is to compare these different scenarios, whenever is needed to distinguish them I will use a capital letter subindex. C will denote the classical unconditional scenario; the fitness function will be denoted as W_C and the allelic level of expression will be specified as x_C and y_C. Similarly, the conditional strategy will be specified by a subindex T (of threat); then, W_T is the fitness function and x_T and $y_T = y(x_T)$ the expression levels under this scenario.

2.2 Fitness

Henceforth, capital letters will refer to functions while lowcase letters will refer to functions evaluations or parameters. For example, let G be a function of two arguments, then g is the value it takes when evaluated at (x, y), this is $g = G(x, y)$. Parental inheritance will be refered with an r (for relatedness) in a general description. When talking exclusivelly about the maternal line $r = m$, and when the paternal line is referred $r = p$. Later on, a function R will be constructed, which will refer to the general case of the parental lines functions M and P, thus, remind that lowcase r is the parameter of relatedness while capital case R is the function.

Visual support will be particularly useful when introducing the maternal threat scenario, therefore some graphical examples will be given throughout the manuscript. Nevertheless, the reader must keep in mind the general analysis since these graphs are just auxiliary and specific solutions depend on choosing particular functions.

2.2.1 Total fitness

The total inclusive fitness of an autosomal allele W is considered to be the weighted average of the contribution by each parental allele, W_m when maternally derived and W_p when paternally derived

$$W = (W_m + W_p)/2$$

alleles are weighted equally as it is considered that in the long term they will be derived half the time from the mother and half the time from the father.

2.2.2 Functional growth

Alleles' gain in fitness is shaped by the functional level of growth promoter (G), that could also be interpreted as the level of demand of the current offspring. G is zero without x production regardless the level of production of y, if any. When $x > 0$, G increases monotonically with x but strictly decreases with y, hence

$$G(x, y) = 0 \text{ for } x = 0; \quad \frac{\partial G}{\partial x}(x, y) \geq 0 \quad \text{and} \quad \frac{\partial G}{\partial y}(x, y) < 0$$

Figure 1 presents two functional forms that G could take, while the example in figure 1a can increase functional growth infinitely with x, the example in figure 1b x will reach a maximum level of functional growth regarless the increase in x expression.

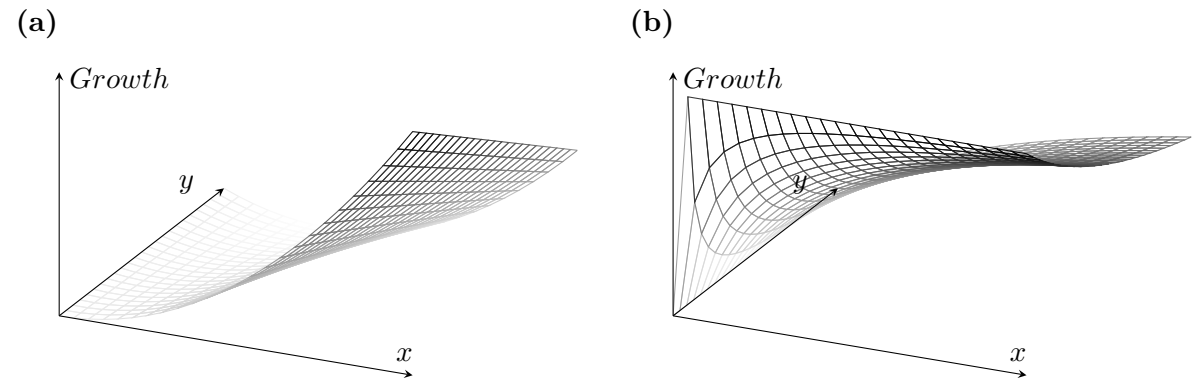

Figure 1: Examples of functional forms for functional growth. (a) $G(x, y) = x(x - \gamma y)$. (b) $G(x, y) = x/(x + \gamma y)$. x is the growth promoter concentration, y is x's inhibitor concentration and γ represents a clearance rate. Darker shade indicates greater value.

2.2.3 Cost of production

Any production of the growth factor and the growth inhibitor has a cost C to alleles' fitness. This means that C strictly increases with both x and y

$$\frac{\partial C}{\partial x}(x, y) > 0 \quad \text{and} \quad \frac{\partial C}{\partial y}(x, y) > 0$$

Figure 2 shows an example of linear costs for both loci.

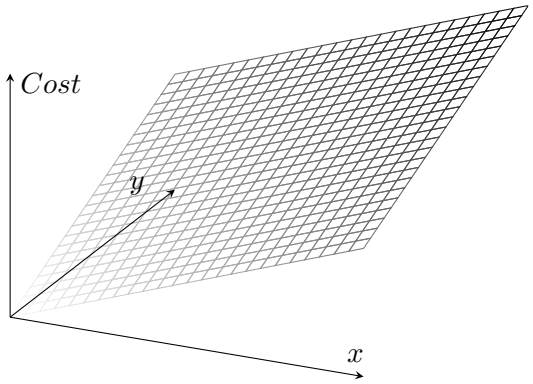

Figure 2: Example of a function of linear costs for both loci. $C(x, y) = \lambda_x x + \lambda_y y$. x is the growth promoter concentration, y is x's inhibitor concentration and λ is the cost of production of the subindexed molecule. Darker shade indicates greater value.

2.2.4 Inclusive fitness

Inclusive fitness W_r is considered to be the effect of the functional expression level g on the allele's direct fitness U, and the expected fraction of copies of the allele r present in the mother's future offspring V, balanced by the associated costs C (see figure 3). Then, the inclusive fitness or reproductive success for a given allele is

$$W_r(x, y) = U[G(x, y)] + rV[G(x, y)] - C(x, y) \tag{3}$$

where r represents maternal (m) or paternal (p) inheritance, thus relatedness.

Direct fitness U is considered to increase monotonically with g, but it is subject to diminishing returns, this means that U saturates (see figure 3a):

$$U'(g) > 0 \qquad \text{if } g_1 < g_2 \text{ then } U(g_1) < U(g_2) \text{ and } U'(g_1) > U'(g_2)$$

Inclusive fitness can increase if the parents reproduce again, but the mother's residual reproductive value V decreases with the individual's level of demand g, as the maternal reserves for future offspring are reduced with increased level of demand of the current offspring, therefore there is a trade off between current and future reproduction for the mother (figure 3b):

$$V'(g) < 0 \qquad \text{if } g_1 < g_2 \text{ then } V(g_1) > V(g_2) \text{ and } V'(g_1) > V'(g_2)$$

If the mother reproduces again, a rare allele in the current offspring will be present in the mother's future offspring in a frequency of 0.5 ($m = 0.5$). Nonetheless, the relatedness to the father is dependent on the mating system, thus $0 \leq p \leq 0.5$. p can also be understood as the probability that the residual reproductive value of the mother is shared with a different male than the father of her current offspring. The sum of direct fitness and inclusive fitness (call it R for the general case) gives the total description of the contribution of the functional level of growth g to fitness (figure 3c). R minimum fitness is the inclusive fitness of zero functional growth level, and R saturates at the same level than U.

$$R(g) = U(g) + rV(g) \qquad min(R) = R(0) = rV(0) \text{ and } max(R) = max(U)$$

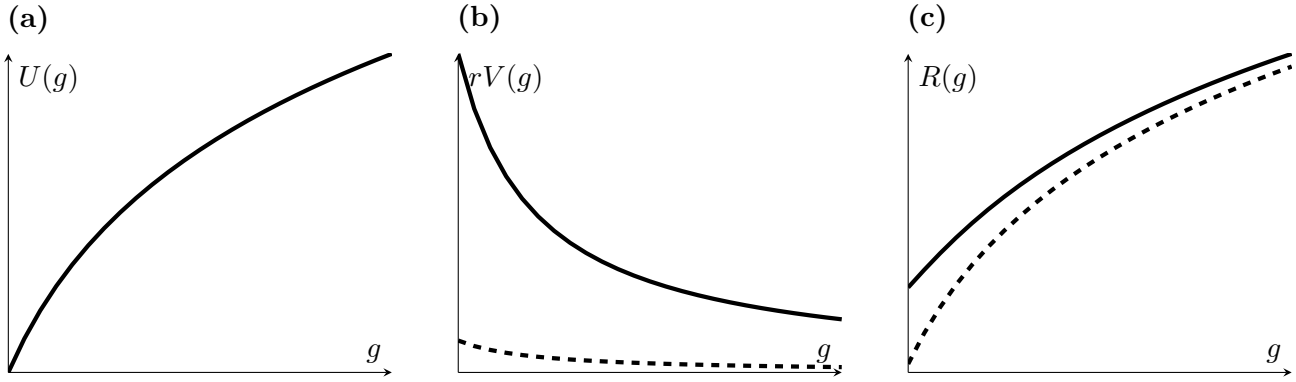

Figure 3: Effect of different paternity in the inclusive fitness. (a) Individual fitness U is an increasing function of g subject to diminishing returns. (b) The maternal residual reproductive value V weighted by the relatedness r to the future offspring. (c) Sum of both fitness components ($R(g) = U(g)+rV(g)$), notice that fitness for $g = 0$, fitness is not zero. Solid line $R = M$ and $r = m = 0.5$, dashed line $R = P$ and $r = p$, here it is assumed $0 < p < 0.5$.

Notice that although G and C have both the same domain, its evaluation to growth $G(x, y)$ and cost $C(x, y)$ cannot be associated ($G \to C$). Figure 4a shows how for a specific functional growth level there is more than one possible associated cost. For a given g there is a minimum cost associated to the minimum amount of x required to attain that level of functional growth ($g = x$ and $y = 0$). Then, the inclusive fitness equation (equation 3) is completed by deducting the specific cost of production of

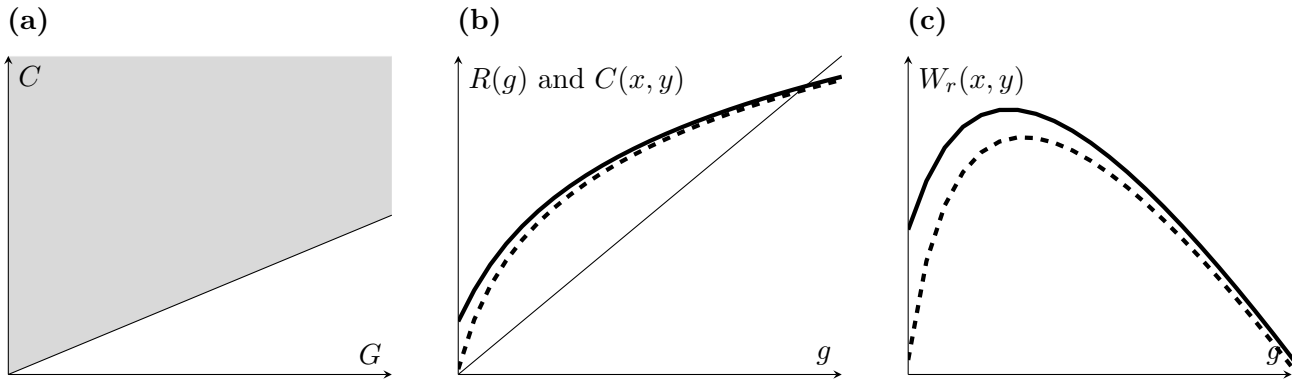

Figure 4: Inclusive fitness construction. (a) Growth-cost relation ($G \to C$). Every functional growth level could be achieved by different (x, y) that are associated to a different cost. (b) A specific growth-cost relation (thin line) is taken as an example to observe the effect of cost on the total increase in inclusive fitness $R(g)$ (in next panel). (c) The cost lowers down the gain in fitness (full fitness equation $U(g) + rV(g) - C(x, y)$ is computed) for large values of functional growth g, therefore fitness has a maximum. Notice that the maximum is different for maternal alleles (solid line) compared to patenal alleles (dashed line).

a given (x, y) to the gain in fitness attained by its level of functional growth (figures 4b and 4c). The cost lowers down fitness for large values of functional growth, since they are associated to higher cost, producing a hump shaped fitness landscape for both alleles with a different maximum for paternal alleles than for maternal alleles (figures 4c).

2.3 Evolutionarily stable strategies

To study the evolution of the expression level at each locus under the different strategies I compare the equilibrium points. This is comparing the level of expression at the evolutionarily stable strategy (ESS) when loci are independent to the ESS under the maternal threat. It is assumed that the change in expression of an allele ξ is sufficiently small to approximate the change in fitness (ΔW) by the partial derivative of the fitness function, or local gradient, with respect to ξ. Then, $\hat{\xi}$ is considered to be an ESS if it occupies a local maximum (Geritz et al., 1998), this means that any change in expression level decreases fitness

$$\left.\frac{\partial W}{\partial \xi}\right|_{\hat{\xi}} = 0; \quad \frac{\partial^2 W}{\partial \xi^2} < 0$$

A rare allele in the growth promoter locus will differ in expression only when it is paternally derived and its expression level will always be zero in the silenced maternal line. Then, it is enough to consider the effects in fitness in the paternal line (W_p) as fitness in the maternal line remains unchanged. Analogous for the growth inhibitor, it is enough to consider the effects on the maternal fitness (W_m). For each scenario, an ESS is any point $\{\hat{x}, \hat{y}\}$ that satisfies joinly the pair of equations describing the rate of change in fitness (local fitness gradient) for each allele equated to zero. This is

$$\partial_1 W_p(\hat{x}, \hat{y}) = 0 \tag{4a}$$
$$\partial_2 W_m(\hat{x}, \hat{y}) = 0 \tag{4b}$$

where ∂_i denotes the partial derivative with respect to the i-th argument of the function.

3 Analytical results

The fitness function W_r itself (equation 3) does not have changes driven by allelic strategies, the difference lies down in the entry of the function. When loci are independent, fitness is evaluated as $W_r(x, y)$, under the contingency of the maternal threat fitness evaluation becomes $W_r(x, y(x))$. As the

entry is different, fitness evaluation changes. It is of great importance the fact that the rate of change in fitness is modified and that the conditional behaviour allows one locus to contribute to the other's fitness because it has repercutions in the stability analysis of the ESS (see equations 4a and 4b). To maintain notation short, henceforth I will drop the dependencies on x and y; then, $W = W(x, y)$, $G = G(x, y)$, $C = C(x, y)$.

3.1 Unresponsive strategy equilibrium

When loci are under independent selective forces (equation 1), each locus changes its expression in a "blind" fashion, until the optimum level of expression for each locus is achieved. The rate of change in fitness under this scenario is

$$\partial_1 W_{Cp} = P'\partial_1 G - \partial_1 C \tag{5a}$$
$$\partial_2 W_{Cm} = M'\partial_2 G - \partial_2 C \tag{5b}$$

where $P' = U'(G) + pV'(G)$ and $M' = U'(G) + mV'(G)$.

If the growth promoter is embebed in an environment of physiologically unresponsive growth inhibitors, then the ESS is any $\{\hat{x}_C, \hat{y}_C\}$, that makes

$$\partial_1 W_{Cp}: \qquad P'\partial_1 G = \partial_1 C \tag{6a}$$
$$\partial_2 W_{Cm}: \qquad M'\partial_2 G = \partial_2 C \tag{6b}$$

3.2 Maternal threat equilibrium

Let $y_s = y(x)$ be a responsive (conditional) strategy. The first intuitive result is that y locus should be able to manipulate its own fitness gain, which does not happen. Nevertheless, while y's rate of change in fitness remains unchanged, the responsive strategy modifies the rate of change in fitness of x's.

$$\partial_1 W_{Tp} = P'\partial_1 G - \partial_1 C + y_s'[P'\partial_2 G - \partial_2 C] \tag{7a}$$
$$\partial_2 W_{Tm} = M'\partial_2 G - \partial_2 C \tag{7b}$$

where $y_s^{'}$ is $\frac{dy_s}{dx}$. For further comparison purposes, let us reduce the analysis to linear functions since the derivative will be known. Notice that the unresponsive scenario is a special case of the maternal threat if y_s is a constant function.

As the maternal threat specifies a relationship between genes, y's response to x's behaviour is now restricted. While under the independent scenario y could explore any possible expression level for a given x expression, under the maternal threat scenario y is restricted to a single line, the image of its specific function (see figure 5). Then, even if y_s cannot modify its own fitness, y_s should force a modified expression of both genes by an unilateral change of x. This results in a change in the marginal change in y's expression, which also contributes to x's fitness.

When growth promoter alleles are facing responsive strategies, their equilibrium point is modified to cope with the extra y input to its dynamics. Then an ESS would be any $\{\hat{x}_T, \hat{y}_T\}$ that satisfies

$$\partial_1 W_{Tp}: \qquad P'[\partial_1 G + y_s'\partial_2 G] = \partial_1 C + y_s'\partial_2 C \tag{8a}$$
$$\partial_2 W_{Tm}: \qquad M'\partial_2 G = \partial_2 C \tag{8b}$$

3.3 Reduction in the expression level at both loci

The first thing to notice is that $\{\hat{x}_C, \hat{y}_C\}$ is not an ESS outside the independent scenario as

$$\partial_1 W_{Tp} = 0 + y_s^{'}[P'\partial_2 G - \partial_2 C] \neq 0$$
$$\partial_2 W_{Tm} = 0$$

$$\text{even more} \qquad y_s^{'}[P'\partial_2 G - \partial_2 C] < M'\partial_2 G - \partial_2 C = 0$$

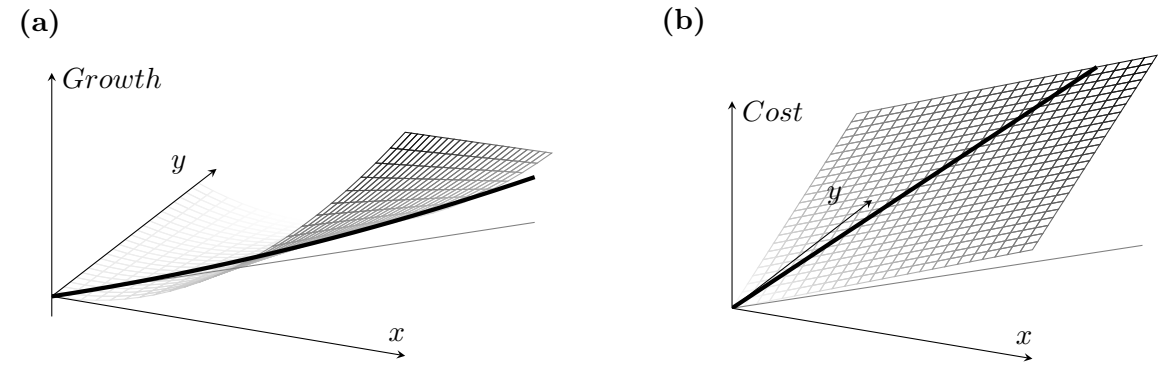

Figure 5: Effect of the functional response of y in the evaluation of growth and cost. A linear y_s is used just as an example (thin line in the XY plane). The thick black line represents its evaluation to the (a) growth and (b) cost surfaces. x is the growth promoter concentration, y is x's inhibitor concentration. Darker shade indicates greater value.

As the selection gradient at $\{\hat{x}_C, \hat{y}_C\}$ is not at equilibrium when the y is using a responsive strategy, the level of expression of the x locus will move towards the point of higher fitness. Knowing that the selection gradient is negative, we can predict that x level of expression will be lower down to search for a new equilibrium that balaces the net effect of y_s contribution.

If a new equilibrium $\{\hat{x}_T, \hat{y}_T\}$ were to exist, it has to satisfy equation 8. This problem can be reduced to only one equation by describing both, the paternal and the maternal fitness, with the maternal dynamics ($\partial_2 G$). To reach equilibrium at both loci, and for any y_s, it is necessary to satisfy

$$P'[\partial_1 G + y_s^{'}\partial_2 G] - \partial_1 C = y_s^{'} M' \partial_2 G \tag{9}$$

since

$$\partial_1 W_{Tp}: \qquad \frac{P'[\partial_1 G + y_s^{'}\partial_2 G] - \partial_1 C}{y_s^{'}} = \partial_2 C$$

$$\partial_2 W_{Tm}: \qquad M'\partial_2 G = \partial_2 C$$

Under the maternal threat selection may promote the reduction in expression at the growth promoter locus, and the new expression level may be stable. Then, we can evaluate the function y_s to know what is the effect of the new x level of expression at the growth inhibitor locus. As y_s is an increasing function, and $x_T < x_C$, then $y(x_T) < y(x_C)$, hence $y_T < y_C$. This shows that if the growth inhibitor is playing a conditional strategy, the reduction of the promoter's expression level drags down the inhibitor's expression level, meaning that both loci would reduce their expression by an unilateral reduction of the growth promoter locus, this is

$$\{\hat{x}_T, \hat{y}_T\} < \{\hat{x}_C, \hat{y}_C\}$$

3.4 Direct and inclusive fitness partition

Equation 7a makes clear that the independent strategy x_{ind} (with a y_s of slope zero) is a special case of the maternal threat. With equation 9 we can see how from the growth promoter perspective, for any y_s with slope higher than zero the cost of production of the inhibitor (y) can be interpreted as a contribution to growth. It also allows to see that at the extreme where y has full control of x's fitness, x behaves as a fixed expression level ($\partial_1 G = \partial_1 C = 0$). The equilibrium could be achieved only by doing $p = m$; a restriction that does not allow the evolution of a responsive strategy.

We can study separately the effects on direct fitness U and inclusive fitness V of the conditional strategy by rewriting equation 9 as $P'\partial_1 G + y_s'(P' - M')\partial_2 G = \partial_1 C$ and using the fact that $P' - M' = (p - m)V'(G)$, then

$$U'(G)\partial_1 G + V'(G)[p\partial_1 G + (p - m)y_s'\partial_2 G] = \partial_1 C \tag{10}$$

Interestingly the conditional strategy y_s does not contribute to direct fitness. Nonetheless, the effect on inclusive fitness is as big as the difference in relatedness. This has direct implications on what type of mating systems can evolve responsive strategies. In mating systems where the relatedness to the mother is on average the same as the relatedness to the father, a responsive strategy will have no effect in fitness and at all times it would be indistinguishable from the independent strategy. The practical effect is that a responsive strategy can only evolve when there is asymmetric relatedness. The larger the asymmetry is, the stronger this effects will be.

Since the the conditional strategy y_s only affects the inclusive fitness component, the maternal threat can be seen as an opportunity to reallocate resources. The independent strategy reaches a very high expression level of the gene products, which may deplete the maternal resources leaving few for future offspring. However, when x locus sees itself in an environment of responsive y alleles (y_s), the reduction in production of gene products, relative to the independent strategy, leaves more resources available for the mother's future offspring.

4 Particular example

To understand better the implications of the maternal threat on the evolution of x's expression level, I compare graphically how different is the behaviour of particular functions G, C, U and V when different linear responsive strategies y_s that pass through the same arbitrary point $\{\bar{x}, \bar{y}\}$ are evaluated. This is asking how would $\bar{x}$ level of expression evolve if it were embebed in different y_s environments. The functions used for this example are in table 1.

Table 1: Functions for the particular example

$$G(x,y) = x(x - (6/10)y)$$
$$C(x,y) = (1/4)x + (3/20)y + 10$$
$$U(G) = 5ln(G+1)$$
$$V(G) = (1/4)(G+1)$$

$$x_{ind}\text{: } y = 2$$
$$y_1 = (1/10)x + (17/10)$$
$$y_2 = x - 1$$
$$y_3 = 3x - 7$$
$$y_4 = 7x - 19$$
$$y_5 = 16x - 46$$
$$y_{ex} = 30x - 88$$

Figure 6 presents a particular example of the functional growth function G (figure 6a) and the cost function (figure 6b) in a surface plot to visualise how different linear y_s strategies would explore these surfaces. To compare how those different y_s modify x's dynamics, the evaluation of (x, y_s) in the functions are plotted against x; G (figure 6c), C (figure 6d), P and W (figure 7). Now, let us notice describe the extreme cases. On the one hand, when x is independent (x_{ind}) it can take any value for a particular constant value of y. (light blue lines in figures 6 and 7). This can be described as a linear y_s with constant expression, or zero slope. On the other hand, if y_s were to evolve an extreme strategy, the dynamics at x locus would be governed exclusivelly by the contribution of y; x would behave as a fixed value, and y_s would have an infinite slope. Although, the analytical results show that this case cannot possibly evolve, very extreme reaccionary functions can y_{ex} (dark blue lines in figures 6 and 7).

4.1 Growth and cost

While C is always an increasing function regardless y_s, G changes its quality from an increasing to a decreasing function according to y'_s and G's isocline is the watershed. Let us call y_{iso} to the functional response such that $G(x, y_{iso})$ is constant for all x and that passes through $\{\bar{x}, \bar{y}\}$. Hence, (x, y_{iso}) is an isocline of G and $\partial_1 G + y'_{iso}\partial_2 G = 0$. Notice though, that the isocline is not a linear function. When the slope of y_s is lower than y'_{iso}, then $\partial_1 G > y'_s \partial_2 G$ thus the higher functional growth is achieved by increasing $\bar{x}$ expression. However, if the slope of y_s is higher than y'_{iso} the inequality changes to

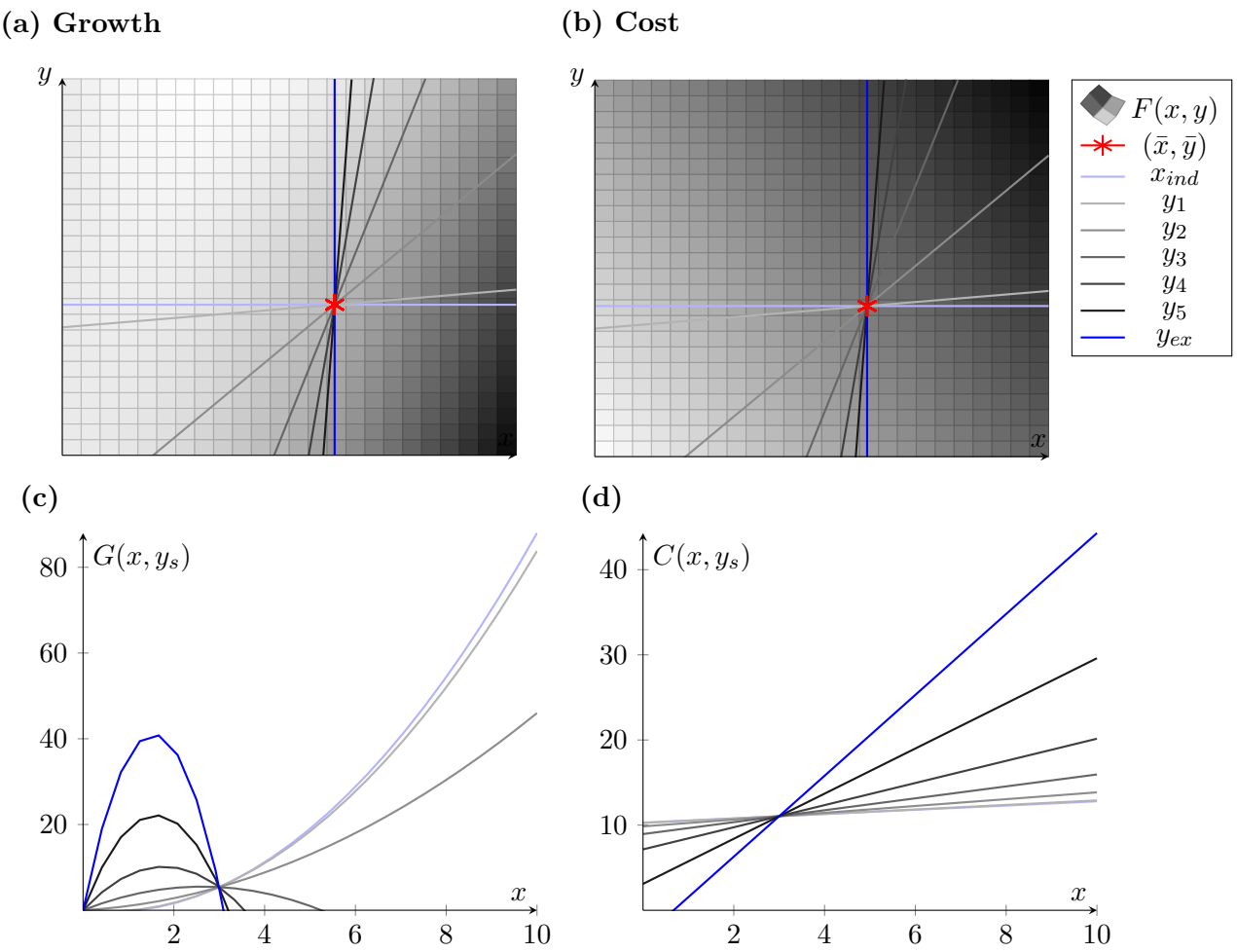

Figure 6: Effect on functional growth and cost of different funcional linear responses y_s. a) Surface plot of functional growth $G(x,y)$ b) Surface plot of cost $C(x,y)$. In both panels the lines are different y_s responses that pass through the same point $(\bar{x},\bar{y})$ (red asterisk). Light blue is x independent (x_{ind}), the shades of gray represent y_s from lower to higher slopes, and dark blue is a y_s with very steep slope. c) Plot of functional growth function G against x. d) Plot of cost function C against x. In table 1 are the the functions udes for this example.

$y_s'\partial_2 G > \partial_1 G$, G becomes a decreasing function and higher functional growth is achieved by lower $\bar{x}$'s levels of expression.

Departing from x_{ind} (increasing the slope of y_s) has two effects on functional growth (figure 6c). Firstly y_s restricts the range of values that x can take. This results is a diminished x expression just because higher values are not attainable. Higher slopes of y_s result in more restricted domains for x which are constrained to low values. The second effect is the modification of the speed of exploration of functional growth by x dynamics. The higher the slope of y_s the faster the dynamics. This means that higher slopes achieve the same (attainable) functional growth at lower levels of x. While x_{ind} will always increase or saturate functional growth with increasing x, higher slopes of y_s will tend to decrease functional growth after a certain critical x value.

At the same time, while C is always a penalisation to fitness, the strength shifts with y_s (figure 6d). With x_{ind} the cost to x's fitness are exclusively those of producing a given level of x, increasing the slope of y_s adds up the cost of producing y at a rate dictated by the slope. Then, the maternal threat increases the penalisation speed. A small increase in x expression will cause a particular increase in y expression, with higher costs for x than if x were independent. However, a small reduction in x expression reduces y's expression too therefore, the cost.

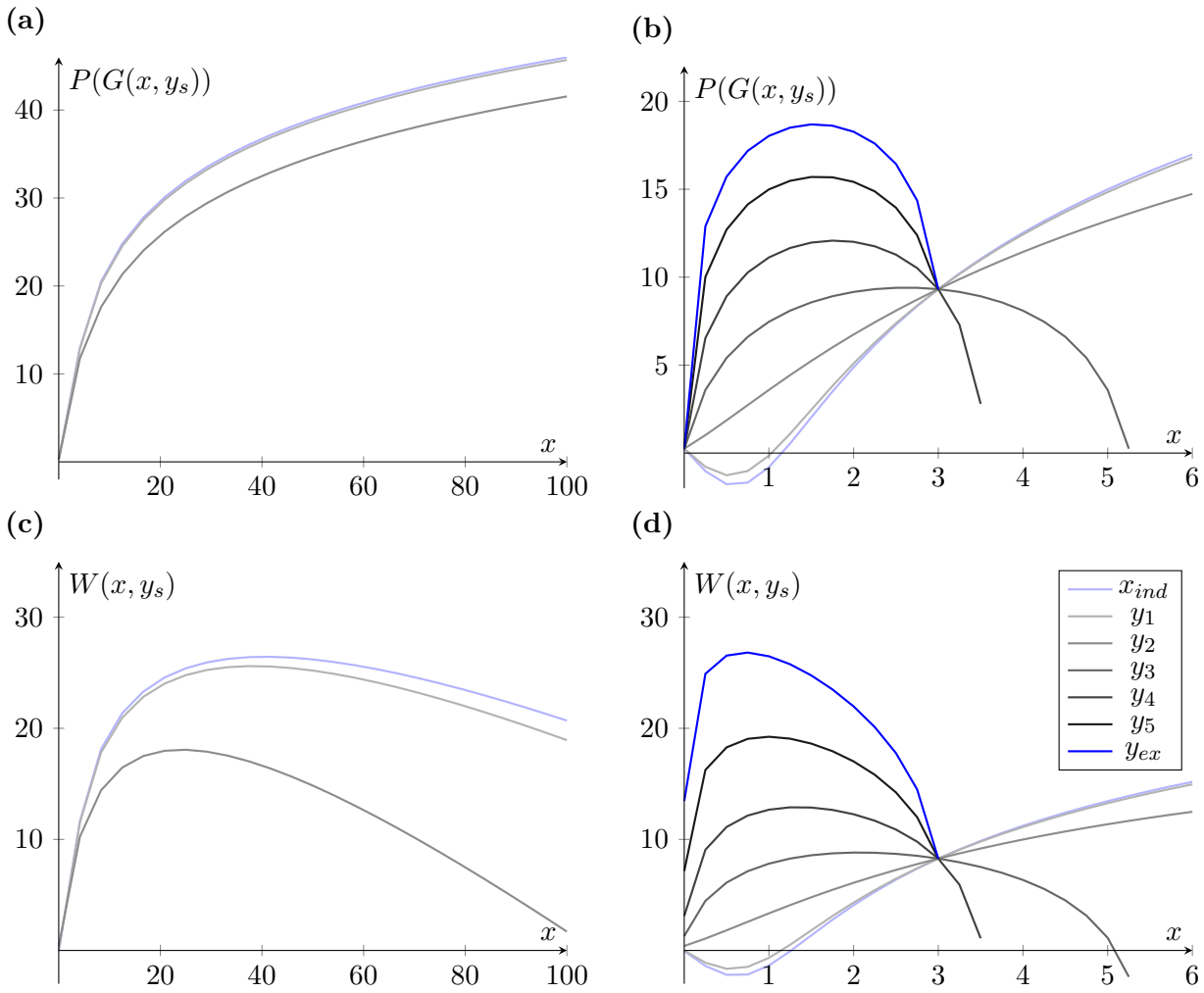

Figure 7: Effect on fitness of different funcional linear responses y_s. a) and b) show plots of the gain in fitness P (no cost deduction) against x.c) and d) show plots of the total inclusive fitness W (with cost deduction) against x. Since low slopes balance out at high levels of growth promoter expression but high slopes restict expression of growth promoter to low levels: a) and c) show a wide range of x while b) and d) show a zoom to low values of x. In table 1 are the the functions udes for this example.

4.2 Fitness and expression level

The modified functional growth and cost affect fitness and, in turn, x's expression level. For x_{ind} low x expression levels have minimum fitness, P (gain in fitness) increases with x until it saturates (figure 7a). Fitness gain and cost balance out at high levels of expression (figure 7c). Sligths departures from x_{ind} (low slopes of y_s) have as main and strongest effect the reduction in gain in fitness for high levels of expression, hence, P saturates at a lower level (figure 7a). This results in lower fitness for the same (high) x level of expression when y is playing a responsive strategy (figure 7c). However, at lower levels of expression, the opposite happens. The same (low) x level of expression increases in fitness with the slope of y_s. With higher slopes the effect of y is strong enough to reshape P. Instead of saturating it inherits the hump shape of G (figure 7b). Then, W has a maximum at lower levels of expression than lower slopes (figure 7d).

5 Discussion

The aim of this work is to assess the evolution of the expression level of antagonistically imprinted loci when one of them is physiologically responsive to the expression level of the other. There are more than

150 imprinted genes in therians and they interact in complicated ways (Gutierrez-Marcos et al., 2012). Previous theoretical work has shown that imprinting can evolve and each locus will increase expression until fitness in its own parental line is optimised (Wilkins and Haig, 2001). Nonetheless, this previous work assumes that each locus is under independent selection, therefore loci are unresponsive. However, it is known that cells have innumerable mechanisms to sense concentrations in their citoplasm and in their surrounding environment. These mechanisms can trigger expression or silence genes at different times or tissues (Costa et al., 2012). If certain genes are promoting or silencing the expression of other genes, we can question the validity of assuming independency between loci. This may be especially true for genes that are part of the same cascade as in the antagonistically imprinted systems $Igf2$-$Igf2r$ and $Dlk1$-$Grb10$.

Recognising that genes may have physiologically relevant interactions motivated this work. Here I study if, once loci are imprinted, the level of expression can be modified and more especifically reduced by imposing a conditional strategy at one of the locus. The conditional strategy was implemented by relaxing the independence assumption in the model of Wilkins and Haig (2001) based on the $Igf2$-$Igf2r$ system. The expression level of $Igf2r$, a maternally expressed growth inhibitor, was considered to be able to condition on the total expression of the growth promoter $Igf2$, which is paternally expressed. This strategy, called maternal threat, does not modify the fitness function. It only implements a relationship between the loci by allowing the expression at the growth inhibitor locus ($Igf2r$) to be a function of the expression level at the growth promoter locus ($Igf2$).

In this work I show how relaxing the assumption of unresponsive gene behaviour does make a difference in the prediction of the expression level that genes will attain. As expected, the independent equilibrium $\{\hat{x}_C, \hat{y}_C\}$ reaches the highest level of expression. Any responsive strategy is able to reduce the expression level at both loci; this is $\{\hat{x}_T, \hat{y}_T\} < \{\hat{x}_C, \hat{y}_C\}$. How the expression level evolves when the growth promoter is immersed within different responsive growth inhibitor environments (y_s) is compared. The result is that extreme y_s strategies achieve lower levels of expression at both loci. This is because the cost of production of the inhibitor can be reinterpreted as a contribution to functional growth if the growth promoter expression is reduced.

The analytical result in equation 10 is twofold. First, it shows that assymetric relatedness is a prerequisite for the contingent strategy to evolve. This is consistent with the construction under the kinship theory, since the analysis was made for already imprinted loci. Therefore, imprinting and any other allelic behaviour derived from it requires a mating system where paternity is uncertain for them to evolve. There is no particular "a priori" reason to expect a change in the mating system. Second, it allows to interpret the final effect of the responsive strategy in fitness terms. Because it is only the inclusive fitness component the one that is affected by the maternal threat and the growth promoter reduces its expression, it is possible to state that current offspring is reducing its level of demand on mother's resources. Therefore, the strategy is able to create a new environment that allows the reallocation of resources to future offspring.

One of the most popular mathematical descriptions for molecules interactions to other products are Hill type functions (Segal and Widom, 2009). These have been used to obtain fairly accurate predictions on gene expression (Segal et al., 2008; He et al., 2010). To describe the interaction of these molecules by a Hill function two parameters are required: the cooperativity coeficient and the dissociation constant. In this case, no information on any of them is available. I reduced the analysis to linear functions to keep a simple and tractable scenario since the slope of the line fully describes the contribution to the other loci fitness. Nevertheless, it is possible to define a vicinity around the inflection point where a Hill like function will look like a linar function. Then only one parameter (the slope) is needed to describe the dynamics, which makes the analysis here presented fully valid in that vicinity.

Another interesting question rising from this study is if a responsive strategy y_s is actually able to evolve. I argue that these is actually the case, because virtually all genes ar subject to regulation which is highly important for accurate development. However, further research in necessary to develop this question in a deeper way, including formal analysis on fitness changes at the growth inhibitor locus, which was beyond the aim of this work. Assuming that the strategy has evolved, it is of great interest to ask what would be uninvadable y_s function. While studying several type of functions may be

insightful, it would represent an unnecessarily complicated work. The reduction to a specific type of functions may allow to study the optimisation of the strategy by assuming that selection acts on the descriptive parameters of the function (Botero et al., 2010). For example, as linear functions have been chosen for this work, it could be possible to study selective pressures on the slope and the intercept. With the current analytical results it is expected that very extreme responses are the most likely to evolve, as they allow for lower production of gene transcripts.

Although we may not know the specific functional response of imprinted genes interactions, it is of great importance to be aware that complicated responses can evolve and have vast implications on their level of expression. In that sense, this work is a first attempt to formally describe how different physiological responsive strategies can modify expression levels in an evolutionary prespective, and it clearly states that physiological responses do make a difference in the expected level of expression.

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