ANTAGONISTIC COEVOLUTION OF TWO IMPRINTED LOCI WITH PLEIOTROPIC EFFECTS

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At a locus subject to genomic imprinting, the expression pattern of an allele depends on its parent of origin. Typically, one allele is expressed while the other is transcriptionally silent, and natural selection at the locus will be driven by the inclusive fitness of the active allele. For some aspects of phenotype, the relevant fitness function differs between maternally and paternally derived alleles, so that maternally and paternally expressed imprinted loci become involved in an intragenomic, interlocus conflict. Here I consider the consequences of such a conflict between loci with pleiotropic effects and show that phenotypes are driven away from their optimal values, resulting in a maladaptive, but selectively favored, evolutionary trajectory. The extent to which the evolutionarily stable state departs from the optimal phenotype depends only linearly on the magnitude of the conflict, but is extremely sensitive to the relationship between the pleiotropic effects of the two loci. Thus, even a small intragenomic conflict can have significant deleterious consequences for multiple aspects of phenotype. This result has potential consequences for our understanding of disease states that occur at high frequency in the population, including several common psychological and behavioral disorders such as schizophrenia, bipolar disorder, major depression, and autism.

KEY WORDS: Epigenetics, gene expression, genomic Imprinting, intragenomic conflict, pleiotropy.

Alleles at an imprinted locus exhibit expression patterns that depend on the allele's parent of origin. In the simplest cases, one of the two alleles is transcriptionally silenced, although in many cases, imprinted loci exhibit tissue- and isoform-specific imprinting (Hayward et al. 1998; Blagitko et al. 2000; Peters and Williamson 2008). The kinship theory of imprinting attributes the evolution of imprinted gene expression to a conflict of interests between the maternally and paternally derived alleles at a locus (Haig 2002; Wilkins and Haig 2003a). That is, the level of gene expression that maximizes the fitness of an allele can differ depending on whether that allele was present in a male or a female in the previous generation. Throughout this paper, I will refer to these parent-of-origin conditional fitnesses as the patrilineal and matrilineal fitness, respectively.

Most empirical and theoretical studies of imprinted genes have focused on their roles in early development, and particularly on their effects on fetal and perinatal growth. Growth effects are clearly central to the selective forces relevant to the evolution of

imprinting, and may be predominantly responsible for the acquisition of imprinted expression by alleles at a previously unimprinted locus (Ùbeda and Wilkins 2008). In mammalian reproduction, where the mother is predominantly responsible for providing resources to the offspring pre- and perinatally, paternally derived alleles favor a higher growth rate (and greater demand on maternal resources) than do maternally derived alleles. As a consequence, at loci where increased expression results in increased growth, imprinted loci become maternally silenced, whereas paternal silencing evolves at loci that act to suppress growth (Mochizuki et al. 1996; Haig 1997; Wilkins and Haig 2001; Mills and Moore 2004).

While most imprinted genes modulate fetal growth, many of these genes are also associated with aspects of phenotype that emerge later in development (Tycko and Morrison 2002). In these cases, the fact of imprinted gene expression has important consequences for the selective forces that act on the expression of alleles and on the function of their products. If, for example, an allele

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is maternally silenced, a mutation altering its expression level will be selected solely on the basis of its effect on the patrilineal fitness.

If we consider a single locus in isolation, this outcome is unproblematic, and most likely indistinguishable from the evolutionary end point that would be reached by an unimprinted locus, because the phenotypes that maximize patrilineal and matrilineal fitness are likely to be quite similar in most cases. However, if we consider a pair of antagonistic loci (e.g., a paternally expressed growth enhancer and a maternally expressed growth suppressor), the simplest model permits no pattern of expression that is evolutionarily stable simultaneously at both loci. Rather, a simple analysis predicts a runaway escalation of expression level with no obvious end point. In practice, the end point will be determined either by mechanistic constraints (as when one of the two loci simply can not produce any more gene product), or by the accumulation of deleterious side effects, which result in the costs of further escalation outweighing the benefits (Kondoh and Higashi 2000; Wilkins and Haig 2001).

These simple models assume that the phenotypic effects of the imprinted loci are confined to a single quantity (such as the abstract notion of "growth"). Most loci, however, have pleiotropic effects. Changes in gene expression at an imprinted locus, therefore, will likely have consequences for multiple aspects of the individual phenotype, including aspects of that phenotype for which there is no conflict between the patrilineal and matrilineal fitness. Here I develop a simple, but general, model of a pair of antagonistic imprinted genes (one paternally expressed and one maternally expressed), where changes in the expression level from either locus has effects on multiple aspects of the individual phenotype. Under a set of approximations valid for small changes in both the expression levels and phenotype values, it is possible to analytically determine the evolutionarily stable state (ESS) of the system, where alleles at both loci are at local fitness maxima with respect to further changes in expression. It is shown that, depending on the nature of the pleiotropic effects associated with the two loci, this ESS can produce significantly reduced individual fitness compared with the optimal phenotype. This model provides a concrete illustration of a system in which natural selection can lead systematically to loss of fitness and the fixation of maladaptive phenotypes.

The Model

Assume that the inclusive fitness of an allele residing within an individual is a smooth function of the location of that individual within the phenotype space. I assume the existence of a unique set of phenotype values that maximizes the patrilineal fitness, and another set that maximizes the matrilineal fitness. These two optima are assumed to be close in the phenotype space, and the analysis

will focus on the local vicinity of these optima. This assumption will allow fitness effects to be reasonably approximated by series expansions around these maxima.

In a sufficiently local region around these maxima, the phenotype of an individual can be decomposed into a number of dimensions that are independent in terms of their fitness effects. Note that this assumption can be made without loss of generality because, in the vicinity of the peak, the fitness contours will be elliptical. The assumption of additive fitness effects amounts to a rotation of the axes in phenotype space to align them with the axes of these ellipses. The phenotypic value along each of these dimensions is indicated by a scalar value $\phi_{(k)}$, where (k) indexes these independent dimensions.

While it is possible to transform the phenotype space such that the fitness effects of variation along each phenotypic axis is additive for a single fitness function, it is not necessarily possible to achieve this simultaneously for both the matrilineal and patrilineal fitness functions. Throughout this analysis, I will assume that such a transformation is possible, which is equivalent to assuming that that axes of the elliptical contours around the matrilineal fitness peak are parallel to the axes of the elliptical contours around the patrilineal peak.

In this region, the patrilineal and matrilineal fitnesses can be approximated as

$$w_p = w_{0,p} - \sum \frac{1}{2} \frac{\partial^2 w_p}{\partial \phi_{(k)}^2} (\phi_{(k)} - \widehat{\phi}_{(k),p})^2 \qquad (1)$$

and

$$w_m = w_{0,m} - \sum \frac{1}{2} \frac{\partial^2 w_m}{\partial \phi_{(k)}^2} (\phi_{(k)} - \widehat{\phi}_{(k),m})^2, \qquad (2)$$

respectively, where $\widehat{\phi}_{(k),p}$ and $\widehat{\phi}_{(k),m}$ represent the phenotype values at the patrilineal and matrilineal fitness maxima. Most of the factors that determine fitness will be shared by maternally and paternally derived alleles, and the differences between the two fitness functions are likely to be subtle. Therefore, for clarity of presentation, I will make the simplifying assumption that the local curvatures of the patrilineal and matrilineal fitness functions are equal ($\partial^2 w_p/\partial \phi_{(k)}^2 = \partial^2 w_m/\partial \phi_{(k)}^2$), as are the maximum fitnesses ($w_{0,p} = w_{0,m} = w_0$). It is straightforward to relax the assumption of equal local curvature, so long as the axes of the fitness contours are parallel for the matrilineal and patrilineal fitness functions. The consequences of relaxing this assumption will be described following presentation of the basic results.

Without loss of generality, I will transform these variables in a way that will simplify notation. First, set the phenotype value in each dimension equal to zero for the phenotype value exactly halfway between the patrilineal and matrilineal optima, such that $\widehat{\phi}_{(k),p} + \widehat{\phi}_{(k),m} = 0$ for all k. Next, rescale the phenotype values such that the curvatures are the same in each dimension, and equal

to one ($\partial^2 w_p/\partial \phi_{(k)}^2 = \partial^2 w_m/\partial \phi_{(k)}^2 = 1$ for all k). Finally, rotate the axes in this phenotype value space so that the patrilineal and matrilineal optimal phenotypes lie along the first axis, arbitrarily choosing this rotation so that the patrilineal optimum has a positive value along this axis.

Throughout the analysis that follows, this first axis, labeled $k = 0$, will be referred to as the axis or dimension of conflict. To simplify notation, I will introduce the term $\alpha \equiv \widehat{\phi}_{(0),p} = -\widehat{\phi}_{(0),m}$, which indexes the magnitude of the conflict along this dimension. Note that in all other phenotype dimensions, the patrilineal and matrilineal fitness functions now share the same optimum ($\widehat{\phi}_{(k),p} = \widehat{\phi}_{(k),m} = 0$ for all $k > 0$). Following these transformations and substitutions, equations (1) and (2) reduce to

$$w_p = w_0 - \frac{1}{2}\left(\phi_{(0)} - \alpha\right)^2 - \sum_{k>0} \frac{1}{2}\phi_{(k)}^2 \qquad (3)$$

and

$$w_m = w_0 - \frac{1}{2}\left(\phi_{(0)} + \alpha\right)^2 - \sum_{k>0} \frac{1}{2}\phi_{(k)}^2. \qquad (4)$$

The dependence of these two fitness functions on the phenotypic axis of conflict ($\phi_{(0)}$) is illustrated in Figure 1.

I assume that the phenotype lies initially at the center of the two optima ($\widehat{\phi}_{(k),p} = \widehat{\phi}_{(k),m} = 0$ for all k), and then consider the evolutionary stability of the system in which two imprinted loci are capable of influencing the phenotype. The analysis will focus on changes in the expression level at each locus, but this can be equivalently thought of as changes in the activity levels of the gene products. Assume that one locus is paternally expressed, at a level X. Because alleles at this locus are exposed to selection only when paternally derived, the selective forces on X will be determined by the patrilineal fitness function in equation (3). The second locus
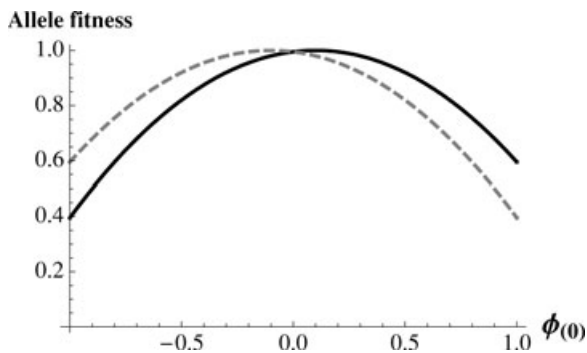

Figure 1. Illustration of the dependence of the matrilineal and patrilineal fitness functions on φ(0) near their maxima. In this example, α = 0.1, so that the patrilineal fitness (solid line) is maximized at the phenotype represented by φ(0) = 0.1, and the matrilineal fitness (dashed line) is maximized at the phenotype φ(0) = −0.1. For all other phenotype dimensions, both fitness maxima occur at φ(k>0) = 0.
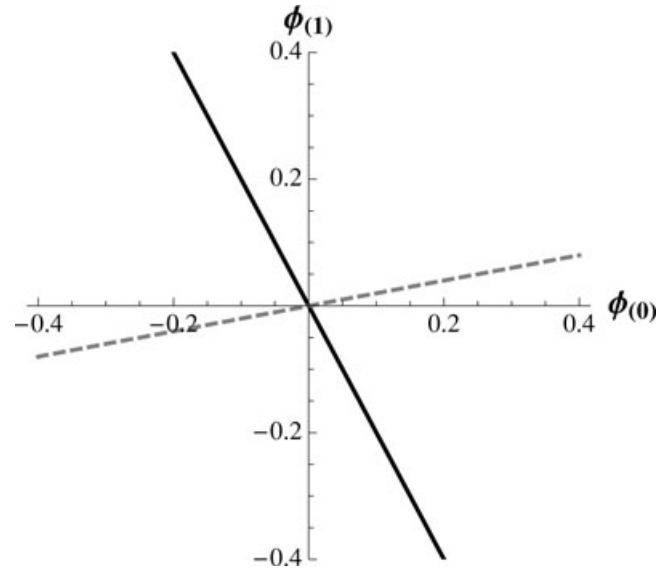
Figure 2. Illustration of the formulation of pleiotropic phenotype effects in the model. The solid line indicates the set of phenotypic effects resulting from changes in gene expression at a locus where β(1) = −2. The dashed line represents a locus with β(1) = 0.2. Note that small absolute values of β mean that the effects of the locus are primarily along the phenotypic axis of conflict. Larger values of β indicate larger effects on other phenotype dimensions.

is maternally expressed at a level Y, and natural selection at this locus will be determined by the matrilineal fitness function in equation (4).

For purposes of this analysis, I assume that the effects of pleiotropy can be approximated by a set of linear effects on each phenotype value (see Fig. 2). For small changes in the expression levels from the two loci (small ΔX and ΔY), this assumption will be a reasonable approximation for a broad set of more realistic pleiotropic effects. Under this assumption, it is possible to derive the phenotype values $\phi_{(k)}$ at the ESS, which will be functions of the pleiotropic effects associated with each of the loci. The consequences of nonlinear and epistatic effects of changes in gene expression level are discussed following the presentation of the results from this linear model.

Analysis of the General Model

Evolutionary stability of the system implies that each locus occupies a local fitness maximum with respect to changes in its level of expression. Thus, the task here is to identify the state of the system where $\partial w_p/\partial X = 0$ and $\partial w_m/\partial Y = 0$ simultaneously. Differentiating equations (3) and (4) and setting each to zero yields the stability conditions

$$(\alpha - \phi_{(0)})\frac{d\phi_{(0)}}{dX} = \sum_{k>0} \phi_{(k)} \frac{d\phi_{(k)}}{dX} \qquad (5)$$

and

$$-(\alpha + \phi_{(0)})\frac{d\phi_{(0)}}{dY} = \sum_{k>0} \phi_{(k)} \frac{d\phi_{(k)}}{dY}. \quad (6)$$

Recalling that the initial state of the system has $\phi_{(k)} = 0$ for all k, the pleiotropic effects of the two loci can be written as

$$\phi_{(k)} = \frac{d\phi_{(k)}}{dX}\Delta X + \frac{d\phi_{(k)}}{dY}\Delta Y. \quad (7)$$

Substituting equation (7) into (5) and (6) yields

$$\alpha\frac{d\phi_{(0)}}{dX} = \Delta X \sum_{k\geq0}\left(\frac{d\phi_{(k)}}{dX}\right)^2 + \Delta Y \sum_{k\geq0}\frac{d\phi_{(k)}}{dX}\frac{d\phi_{(k)}}{dY} \quad (8)$$

and

$$-\alpha\frac{\partial\phi_{(0)}}{\partial Y} = \Delta X \sum_{k\geq0}\frac{\partial\phi_{(k)}}{\partial X}\frac{\partial\phi_{(k)}}{\partial Y} + \Delta Y \sum_{k\geq0}\left(\frac{\partial\phi_{(k)}}{\partial Y}\right)^2. \quad (9)$$

Then, solving equations (8) and (9) simultaneously yields expressions for the equilibrium values of ΔX and ΔY:

$$\Delta X = \alpha\frac{\dfrac{d\phi_{(0)}}{dY}\displaystyle\sum_{k\geq0}\frac{d\phi_{(k)}}{dX}\frac{d\phi_{(k)}}{dY} + \dfrac{d\phi_{(0)}}{dX}\displaystyle\sum_{k\geq0}\left(\frac{d\phi_{(k)}}{dY}\right)^2}{\displaystyle\sum_{k\geq0}\left(\frac{d\phi_{(k)}}{dX}\right)^2\sum_{k\geq0}\left(\frac{d\phi_{(k)}}{dY}\right)^2 - \left(\sum_{k\geq0}\frac{d\phi_{(k)}}{dX}\frac{d\phi_{(k)}}{dY}\right)^2} \quad (10)$$

and

$$\Delta Y = \alpha\frac{\dfrac{d\phi_{(0)}}{dX}\displaystyle\sum_{k\geq0}\frac{d\phi_{(k)}}{dX}\frac{d\phi_{(k)}}{dY} + \dfrac{d\phi_{(0)}}{dY}\displaystyle\sum_{k\geq0}\left(\frac{d\phi_{(k)}}{dX}\right)^2}{\left(\displaystyle\sum_{k\geq0}\frac{d\phi_{(k)}}{dX}\frac{d\phi_{(k)}}{dY}\right)^2 - \displaystyle\sum_{k\geq0}\left(\frac{d\phi_{(k)}}{dX}\right)^2\sum_{k\geq0}\left(\frac{d\phi_{(k)}}{dY}\right)^2}. \quad (11)$$

It is convenient at this point to further simplify the notation by introducing the definitions

$$\beta_{(k)X} \equiv \frac{d\phi_{(k)}/dX}{d\phi_{(0)}/dX}; \quad \beta_{(k)Y} \equiv \frac{d\phi_{(k)}/dY}{d\phi_{(0)}/dY}. \quad (12)$$

The vector of terms $\beta_{(k)X}$ represents the set of linear pleiotropic constraints acting on the X locus. Note that the first of these terms, $\beta_{(0)X}$ is equal to one by definition. Each of the other values of k represents the magnitude of the effect of changes in gene expression along the kth phenotypic axis relative to its effect on the axis of conflict ($k = 0$). A very large value of $\beta_{(k)X}$ would reflect a gene whose primary effect was on some other (nonconflicted) aspect of the phenotype. Very small values of $\beta_{(k)X}$ for all $k > 0$ would indicate a locus that primarily influences the phenotypic axis of conflict, with only minor consequences for other traits.

The introduction of these β terms allows equations (10) and (11) to be rewritten as

$$\Delta X = \frac{\alpha}{d\phi_{(0)}/dX}\frac{\displaystyle\sum_{k\geq0}\beta_{(k)X}\beta_{(k)Y} + \sum_{k\geq0}\beta_{(k)Y}^2}{\displaystyle\sum_{k\geq0}\beta_{(k)X}^2\sum_{k\geq0}\beta_{(k)Y}^2 - \left(\sum_{k\geq0}\beta_{(k)X}\beta_{(k)Y}\right)^2} \quad (13)$$

and

$$\Delta Y = -\frac{\alpha}{d\phi_{(0)}/dY}\frac{\displaystyle\sum_{k\geq0}\beta_{(k)X}\beta_{(k)Y} + \sum_{k\geq0}\beta_{(k)X}^2}{\displaystyle\sum_{k\geq0}\beta_{(k)X}^2\sum_{k\geq0}\beta_{(k)Y}^2 - \left(\sum_{k\geq0}\beta_{(k)X}\beta_{(k)Y}\right)^2}. \quad (14)$$

Substituting these two expressions back into equation (7) yields the evolutionarily stable phenotype values of the system.

$$\phi_{(q)} = \alpha\frac{\displaystyle\sum_{k\geq0}(\beta_{(q)X}\beta_{(k)Y} - \beta_{(q)Y}\beta_{(k)X})(\beta_{(k)X} + \beta_{(k)Y})}{\displaystyle\sum_{k\geq0}\beta_{(k)X}^2\sum_{k\geq0}\beta_{(k)Y}^2 - \left(\sum_{k\geq0}\beta_{(k)X}\beta_{(k)Y}\right)^2}. \quad (15)$$

In equation (15), the index (q) has been introduced for a single phenotype dimension to distinguish it from the (k) index used in the summation terms. Along the axis of conflict ($q = 0$), this expression can be further reduced, recalling that $\beta_{(0)X} = \beta_{(0)Y} = 1$:

$$\phi_{(0)} = \alpha\frac{\displaystyle\sum_{k\geq0}(\beta_{(k)Y}^2 - \beta_{(k)X}^2)}{\displaystyle\sum_{k\geq0}\beta_{(k)X}^2\sum_{k\geq0}\beta_{(k)Y}^2 - \left(\sum_{k\geq0}\beta_{(k)X}\beta_{(k)Y}\right)^2}. \quad (16)$$

The nature of the ESS phenotype values is illustrated geometrically for the two-dimensional case in Figure 3. Recall that the phenotype space has been transformed such that lines of constant matrilineal and patrilineal fitness are circles centered on the matrilineal and patrilineal optima, respectively. The values of $\beta_{(1)X}$ and $\beta_{(1)Y}$ indicate the slope of the vector along which the two loci can alter the phenotype. The system is at equilibrium at the point where neither locus can increase its fitness by shifting the phenotype along the direction determined by its pleiotropic constraints. At the ESS phenotype, a line of slope $\beta_{(1)X}$ (the patrilineal pleiotropic constraint) is tangent to a circle centered on the patrilineal fitness optimum. At the same time, a line of slope $\beta_{(1)Y}$ must be tangent to a circle centered on the matrilineal optimum. Simultaneously satisfying these two criteria uniquely determines the ESS phenotype.
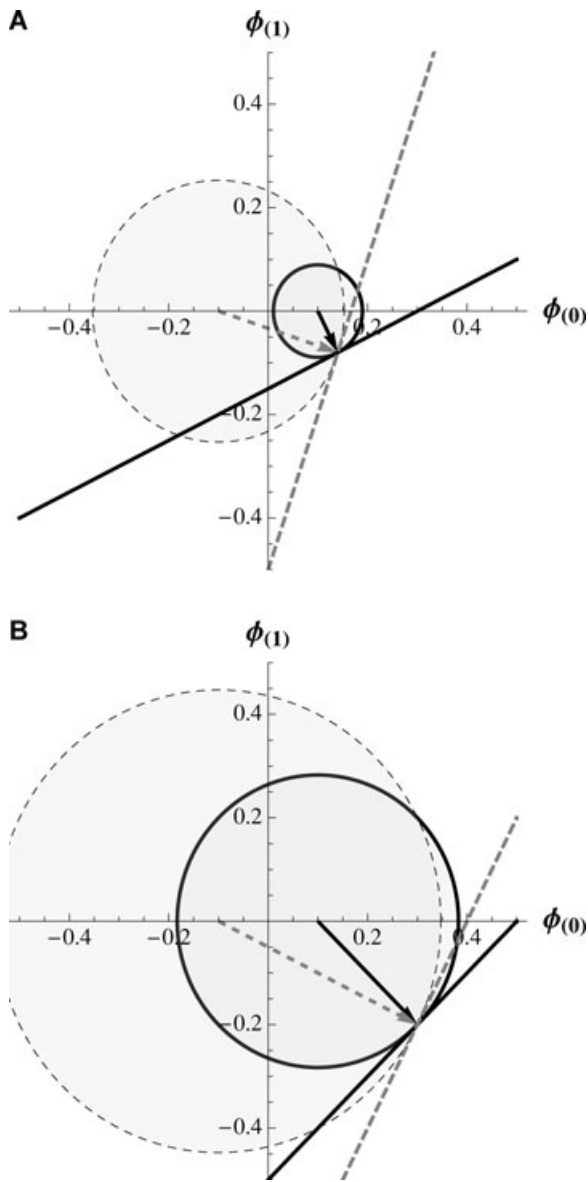
Figure 3. Illustration of the evolutionarily stable phenotype values. The location of the ESS is determined by the nature of the pleiotropic constraints affecting the two loci. In (A) and (B) $\alpha = 0.1$, so that the patrilineal optimum is at $(0.1, 0)$ and the matrilineal optimum is at $(−0.1, 0)$. In (A) $\beta_{(1)X} = 0.5$, and $\beta_{(1)Y} = 3$. Following equations (17) and (18), the corresponding ESS is at $(0.14, −0.08)$. The solid line with slope 0.5, indicating the pleiotropic constraints on the paternally expressed locus, is tangent to a circle centered on the patrilineal optimum. The dashed line, with slope 3, represents the constraints on the maternally expressed locus, and is tangent to a circle centered on the matrilineal optimum. (B) illustrates the case in which the two pleiotropic constraints are more closely aligned, here $\beta_{(1)X} = 1$ and $\beta_{(1)Y} = 2$. The ESS phenotype in this case is further from the origin, at $(0.3, 0.2)$, but again lies at the point where lines with slopes 1 and 2 are tangent to circles centered on the patrilineal and matrilineal optimal phenotype values, respectively.

Interpretation of the Results of the General Model

It is clear from the form of equations (15) and (16) that the magnitude of the phenotypic deviation from the starting point has a simple linear dependence on α, which indicates the magnitude of the intragenomic conflict. The dependence on the β terms, however, is more complicated and potentially much more significant. Note in particular the difference term in the denominator. Because this term can be small, there will be combinations of the β terms that can lead to large phenotype deviations. In particular, inspection of equation (16) suggests that it is possible for $\phi_{(0)}$ to be greater than α or less than $−\alpha$, meaning that the evolutionarily stable phenotype value can be more extreme than the values favored by natural selection acting at either of the loci involved.

Similarly, if we consider the phenotype values for the dimensions that are not the target of the intragenomic conflict, equation (15) makes it clear that these phenotype values will equal zero only in very specific circumstances. In general, these other phenotype values will be driven away from their initial conditions. Recall that in each of these dimensions, the matrilineal and patrilineal fitness are both maximized when these phenotype values are equal to zero.

The denominator of (15) and (16) will always be nonnegative. It will be equal to zero only for the special case in which $\beta_{(k)X} = \beta_{(k)Y}$ for all k. Intuitively, this suggests that the magnitude of the departures of the phenotype from optimality depends most strongly on the relationship between the mechanisms of action of the two gene products. The more similar the two are in terms of their pleiotropic effects, the more severe the predicted departure from optimality.

In introducing the model, I made the simplifying assumption that the second derivative of the fitness with respect to changes in the phenotype was identical for the matrilineal and patrilineal fitness functions along each of the phenotypic axes. It is straightforward to relax this assumption, so long as we maintain additivity of the fitness effects for both fitness functions. For simplicity of notation, I will introduce a set of terms analogous to the β terms:

$$\gamma_{(k)X} \equiv \frac{\partial^2 w_p / \partial \phi_{(k)}^2}{\partial^2 w_p / \partial \phi_{(0)}^2}; \quad \gamma_{(k)Y} \equiv \frac{\partial^2 w_m / \partial \phi_{(k)}^2}{\partial^2 w_m / \partial \phi_{(0)}^2}. \tag{17}$$

Using this notation, the expression for the location of the equilibrium phenotype given in (15) becomes

$$\phi_{(q)} = \alpha \frac{\sum_{k \geq 0} \left(\beta_{(q)X} \beta_{(k)Y} - \beta_{(q)Y} \beta_{(k)X} \right) \left(\gamma_{(k)X} \beta_{(k)X} + \gamma_{(k)Y} \beta_{(k)Y} \right)}{\left(\sum_{k \geq 0} \gamma_{(k)X} \beta_{(k)X}^2 \right) \left(\sum_{k \geq 0} \gamma_{(k)Y} \beta_{(k)Y}^2 \right) - \left(\sum_{k \geq 0} \gamma_{(k)X} \beta_{(k)X} \beta_{(k)Y} \right) \left(\sum_{k \geq 0} \gamma_{(k)Y} \beta_{(k)X} \beta_{(k)Y} \right)}. \tag{18}$$

Along the axis of conflict (analogous to (16), above), this reduces to

$$\phi_{(0)}$$

$$= \alpha \frac{\sum\limits_{k \geq 0} \left(\beta_{(k)Y} - \beta_{(k)X}\right)\left(\gamma_{(k)X}\beta_{(k)X} + \gamma_{(k)Y}\beta_{(k)Y}\right)}{\left(\sum\limits_{k \geq 0} \gamma_{(k)X}\beta_{(k)X}^2\right)\left(\sum\limits_{k \geq 0} \gamma_{(k)Y}\beta_{(k)Y}^2\right) - \left(\sum\limits_{k \geq 0} \gamma_{(k)X}\beta_{(k)X}\beta_{(k)Y}\right)\left(\sum\limits_{k \geq 0} \gamma_{(k)Y}\beta_{(k)X}\beta_{(k)Y}\right)}.$$

$$(19)$$

Qualitatively, the effect of increasing one of the γ terms is similar to increasing the magnitude of the corresponding β term. That is, if the curvature in the fitness function is greater in one direction, this increases the extent of the pleiotropic constraint imposed by that aspect of the phenotype.

Two Phenotype Dimensions

To better illustrate the implications of the results derived in the previous sections, I will explicitly consider the special case in which the phenotypic effects of the two loci are restricted to just two dimensions. Equations (15) and (16) then reduce to

$$\phi_{(0)} = \alpha \frac{\beta_{(1)Y} + \beta_{(1)X}}{\beta_{(1)Y} - \beta_{(1)X}} \qquad (20)$$

and

$$\phi_{(1)} = \frac{2\,\alpha}{\beta_{(1)X} - \beta_{(1)Y}}. \qquad (21)$$

The dependence of these phenotype values on $\beta_{(1)X}$ and $\beta_{(1)Y}$ is illustrated in Figure 4. It is easy to substitute these values back into equations (3) and (4) to determine the matrilineal and patrilineal fitnesses associated with this phenotype:

$$w_p = w_0 - 2\,\alpha^2 \frac{1 + \beta_{(1)X}^2}{(\beta_{(1)X} - \beta_{(1)Y})^2} \qquad (22)$$

and

$$w_m = w_0 - 2\,\alpha^2 \frac{1 + \beta_{(1)Y}^2}{(\beta_{(1)X} - \beta_{(1)Y})^2}. \qquad (23)$$

The long-term fitness of an allele at this evolutionarily stable point is simply the average of the patrilineal and matrilineal fitness values:

$$w = 1 - \alpha^2 \frac{2 + \beta_{(1)X}^2 + \beta_{(1)Y}^2}{(\beta_{(1)X} - \beta_{(1)Y})^2}. \qquad (24)$$

The fitness function in equation (24) is plotted in Figure 5.

Large Departures in Phenotype Values and Expression Levels

The conclusions presented are not expected to hold quantitatively for large departures of $\phi_{(k)}$ from zero. Recall that the specific
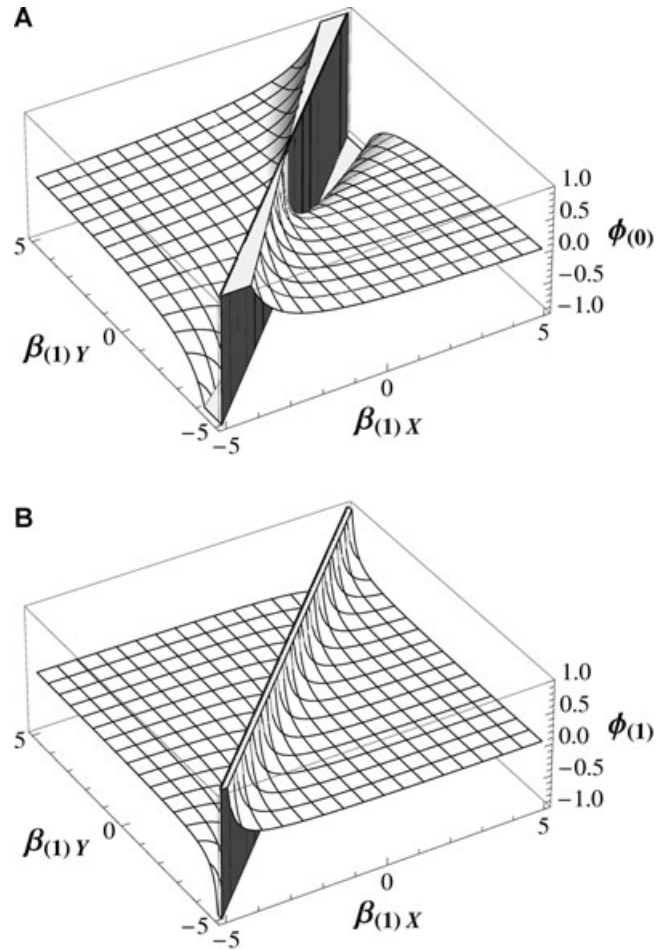

Figure 4. Equlibrium phenotype values as a function of pleiotropic constraints. The magnitude of the conflict, α, is 0.1, as in previous figures. The most extreme phenotype values arise when $\beta_{(1)X}$ and $\beta_{(1)Y}$ are similar. (A) the equilibrium phenotype value along the axis of conflict $\phi_{(0)}$. The absolute value of $\phi_{(0)}$ is greater than α whenever $\beta_{(1)X}$ and $\beta_{(1)Y}$ have the same sign. Note that the vertical range in the figure is $-1.0 < f(0) < 1.0$, and the regions of the plot that are cropped represent cases in which the equilibrium value of $\phi_{(0)}$ has an absolute value of greater than 10 times α. (B) the analogous plot for the other phenotype value $\phi_{(1)}$. The absolute value of $\phi_{(1)}$ is greater than α when $|\beta_{(1)X} - \beta_{(1)Y}| < 2$.

forms used for the fitness functions were derived from an assumption that we are considering phenotype values close zero, where fitness is maximized, and therefore that we could approximate the fitness function using only its second derivative. Similarly, the formulation of pleiotropy has been limited to a set of linear relationships between changes in gene expression and phenotype values. As with the fitness approximation, this approximation should be valid for a broad class of actual pleiotropic effects, but only for relatively small changes in expression.

The analysis presented here suggests particular combinations of values of the $\beta_{(k)X}$ and $\beta_{(k)Y}$ terms that will lead to large
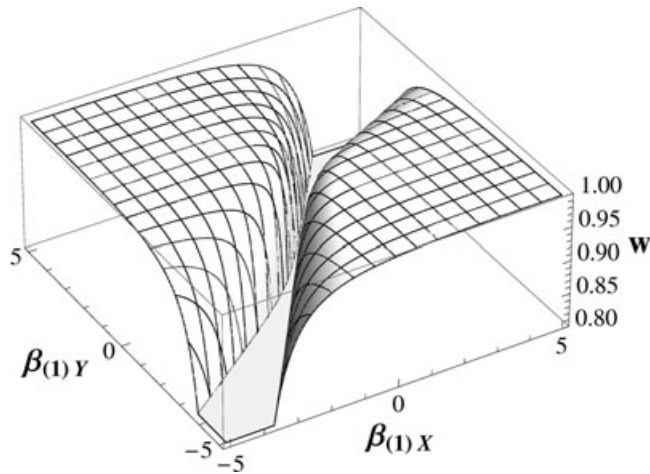
Figure 5. Equilibrium fitness values as a function of pleiotropic constraints. This plot shows the allele fitnesses at the ESS. The magnitude of the conflict α is 0.1 as before, and these fitnesses correspond to the phenotype values shown in Figures 4A,B. As was the case with the phenotype values, the most sever fitness effects are seen when $\beta_{(1)X}$ and $\beta_{(2)Y}$ are similar.

deviations in $\phi_{(k)}$, w, ΔX, and ΔY from their initial values. In these regions, the approximations invoked above are expected to fail, and the exact location of the ESS in phenotype space will be determined primarily by other features of the fitness function, by nonlinearities in the pleiotropic relationships, and/or by epistatic interactions between the two loci.

While real systems will not conform quantitatively to the conclusions of the previous section under these large deviations, the qualitative conclusions of the analysis may be valid under fairly general terms. Specifically, the analysis shows that the presence of two antagonistically coevolving imprinted loci, phenotype values can be driven systematically away from the values favored by both loci. This conclusion applies not only to phenotype values that are the target of the intragenomic conflict, but also to phenotype values over which no conflict exists, but that are mechanistically connected to those loci. The analysis suggests that these departures from optimality can be large, and are strongly dependent on the relationship between the pleiotropic constraints at the two loci. The more closely aligned the constraints are at the two loci, the larger the expected departure from optimality.

Multiple Antagonistically Imprinted Loci

The preceding analysis has been limited to the case of a single pair of antagonistic imprinted loci. It seems reasonable to assume that many phenotypes are influenced by more than two imprinted loci. However, in general, a system with multiple antagonistic imprinted loci will not admit ESS solutions of the sort developed in the preceding sections.

The pleiotropic constraints in a multilocus system can be written in the form

$$\phi_{(k)} = \sum_i \frac{d\phi_{(k)}}{dX_i} \Delta X_i + \sum_j \frac{d\phi_{(k)}}{dY_j} \Delta Y_j, \qquad (25)$$

where i and j index over the paternally and maternally expressed loci, respectively. The equilibrium conditions are

$$\frac{\partial w_p}{\partial X_i} = \alpha \frac{\partial \phi_{(0)}}{\partial X_i} - \sum_{k \geq 0} \phi_{(k)} \frac{\partial \phi_{(k)}}{\partial X_i} = 0 \qquad (26)$$

and

$$\frac{\partial w_m}{\partial Y_j} = -\alpha \frac{\partial \phi_{(0)}}{\partial Y_j} - \sum_{k \geq 0} \phi_{(k)} \frac{\partial \phi_{(k)}}{\partial Y_j} = 0 \qquad (27)$$

for all i and j. Combining these conditions yields

$$\alpha \frac{\partial \phi_{(0)}}{\partial X_s} = \sum_{k \geq 0} \frac{\partial \phi_{(k)}}{\partial X_s} \left(\sum_i \frac{\partial \phi_{(k)}}{\partial X_i} \Delta X_i + \sum_j \frac{\partial \phi_{(k)}}{\partial Y_j} \Delta Y_j \right)$$

$$(28)$$

and

$$-\alpha \frac{\partial \phi_{(0)}}{\partial Y_t} = \sum_{k \geq 0} \frac{\partial \phi_{(k)}}{\partial Y_t} \left(\sum_i \frac{\partial \phi_{(k)}}{\partial X_i} \Delta X_i + \sum_j \frac{\partial \phi_{(k)}}{\partial Y_j} \Delta Y_j \right).$$

$$(29)$$

The indices s and t are introduced here to indicate individual paternally and maternally expressed loci, respectively. The i and j indices are used for summation over all loci. Note that the expression within the parentheses is identical for all of the equations indicated by (28) and (29). Therefore, the only way that all of these equations can be satisfied simultaneously is if every paternally expressed gene shares an identical set of pleiotropic effects, and likewise for the set of maternally expressed genes. In that special case, the analysis reduces to the two-locus case studied above.

This does not imply the absence of an ESS in systems involving more than two loci. Rather, the location of the ESS, if it exists, will depend on higher order terms that have been dropped in the approximations invoked here, similar to what is expected for large deviations in the two-locus case. It is also possible that for certain systems, no ESS will exist even after these effects have been accounted for. Analysis of such systems will require the explicit incorporation of evolutionary dynamics that is beyond the scope of the present paper, but will be the subject of future work.

The Costs of Complexity and Imprinting

The model analyzed here includes both pleiotropy and intragenomic conflict. One of the primary conclusions of the analysis is

that, depending on the exact relationship between the pleiotropic constraints at the two loci, natural selection can drive phenotypes away from the values that maximize fitness. This suggests a fitness cost that pertains to any system with both pleiotropy and conflict.

There is a significant literature addressing the question of evolutionary costs associated with pleiotropy, often termed the "cost of complexity" (Wagner 1988; Orr 2000). While these analyses bear some superficial similarity to the cost suggested here, the nature of the two "costs" is quite different. In particular, the cost of complexity associated with pleiotropy is typically conceived as limiting the availability of beneficial mutations during adaptive evolution. In this case, the cost is in the form of a reduced individual fitness of the equilibrium phenotype relative to the phenotype of maximum fitness.

The analysis here is more relevant to previous discussions of the "cost of imprinting." Typically, this cost is imagined as deriving from the fact of monoallelic expression, or, "foregoing the advantages of diploidy." In particular, discussions of the cost of imprinting often cite the exposure of deleterious recessive mutations. However, the magnitude of such a cost is likely to be small (Spencer and Williams 1997), and may not apply at all to the subset of loci that are most likely to become imprinted (Ùbeda and Wilkins 2008).

The cost associated with imprinting in this analysis has the potential to be large in particular cases, depending on the precise values of $\beta_{(k)X}$ and $\beta_{(k)Y}$. However, this does not imply a barrier to the evolution of imprinted gene expression at these loci. The model analyzed here does not address the evolutionary transition from unimprinted to imprinted gene expression, simply the consequences of selection acting on loci that are already imprinted. In order for the fitness costs described here to select against imprinted gene expression, one would need to invoke a model with higher level selection (e.g., at the species level).

A separate question raised by the analysis is the effect of intragenomic conflict on the evolution of pleiotropy and its effect on the accumulation of complexity. The effect on the evolution of pleiotropy is likely to play a key role in the system of multiple antagonistic loci discussed in the previous section. That solution indicated that the system would not have an ESS unless the pleiotropic effects were identical for all loci with the same gene expression pattern. That is, an ESS is possible if

$$\frac{\partial \phi_{(k)}/\partial X_i}{\partial \phi_{(0)}/\partial X_i} = \frac{\partial \phi_{(k)}/\partial X_j}{\partial \phi_{(0)}/\partial X_j} \qquad (30)$$

for all pairs of paternally expressed genes i and j, and analogous relationships hold among the maternally expressed genes. While this will not generally be the case, if we consider the nonlinear-

ities and epistatic effects on the pleiotropy constraints affecting these loci, there may be particular combinations of expression levels for which (30) and the analogous conditions for the Y loci hold. Thus, in situations where the system arrives at an ESS, the pleiotropic constraints at a locus will evolve to be concordant with the constraints acting on the other loci with which it shares a fitness function.

Conflict between Imprinted and Unimprinted Loci or between Cell Types

The analysis presented here has been framed in terms of a conflict between paternally and maternally expressed loci. Similar reasoning applies to a conflict between imprinted and unimprinted loci, but with the magnitude of the conflict being only half of what it is the case of two imprinted loci. Roughly speaking, the qualitative conclusions presented here should hold, but the perturbation of the ESS phenotype values (which are proportional to α) reduced by a factor of two, and the fitness cost (which is proportional to α^2) reduced by a factor of four.

Previous analysis has shown that tissue-specific reactivation of the silenced allele at an imprinted locus is expected when the matrilineal-patrilineal conflict over expression is reversed in a particular tissue (Wilkins 2006). When cell types differ in their imprinting status at a particular locus, this suggests that expression from that locus has antagonistic phenotypic effects in the two cell types. The result is a conflict between the imprinted and unimprinted expression levels in the two cell types. The analysis developed here would then apply to the arms race between two different aspects of expression from the same locus.

Behavioral Effects and Common Diseases

Perhaps the systems that are best described by the model analyzed here are those imprinted genes that are expressed in the brain, and that have effects on cognition and behavior. Most of these genes also exhibit growth effects in early development, and it is likely that these growth effects are primarily responsible for the acquisition of imprinted expression at these loci. In the absence of specific selection to reactivate gene expression from the silenced allele, the imprinted expression pattern established in early development will be inherited by the brain tissues (Wilkins 2005, 2006).

A wealth of molecular and clinical evidence suggests that many of these imprinted genes do influence cognitive and behavioral phenotypes (Davies et al. 2008a,b; Goos and Ragsdale 2008), and many of these effects are likely to be pleiotropic. The analysis presented here would apply to any behavior for which

there is a difference between the matrilineal and patrilineal optimal phenotypes. There is evidence to suggest that there are, in fact, intragenomic conflicts over particular behaviors, including postnatal feeding behaviors (Haig and Wharton 2003; Ùbeda 2008), maternal care (Lefebvre et al. 1998; Li et al. 1999; Wilkins and Haig 2003b), and reactivity to novel environments (Plagge et al. 2005).

Depending of the exact nature of the pleiotropic constraints acting on the loci involved, the ensuing arms race could result in significant departures from optimal behavioral phenotypes. This could produce systematically maladaptive phenotypes both in behaviors that are subject to an intragenomic conflict and those that are not. In recent years, a number of major psychological disorders, including schizophrenia, bipolar disorder, major depression, and autism, have been associated with variations at imprinted loci (De Luca et al. 2007; Shelton 2007; Crespi 2008; Goos and Ragsdale 2008). Each of these disorders presents an evolutionary puzzle, because, considering their devastating effects, they occur at surprisingly high frequencies in the population. However, the intuition that severely deleterious disorders should be limited to frequencies on the order of 10^{-4} or 10^{-5} is based on an optimizing notion of natural selection. The analysis presented here indicates that this intuition should not be expected to hold for disease states that are influenced by antagonistically coevolving imprinted genes. It may be that the high frequency at which these disorders are found in the population reflects the pleiotropic effects of imprinted gene expression in the brain.

Finally, the results presented here raise a possible complication in the interpretation of the phenotypes of knockouts of imprinted genes. In standard models of intragenomic conflict, where the focus is on a single aspect of the phenotype, qualitative predictions of the consequences of eliminating an imprinted gene are straightforward. Knocking out a paternally expressed locus will shift the phenotype in the direction of the matrilineal optimum, and vice-versa for a maternal knockout (although these predictions are often hard to test in practice because of "overshoot," where the knockout phenotype moves far beyond the optima). The model developed here makes similar predictions regarding the phenotypes of knockouts with respect to the phenotypic axis of conflict, $\phi_{(0)}$: loss of the maternally expressed gene will shift $\phi_{(0)}$ in the positive direction, while loss of the paternally expressed gene will reduce $\phi_{(0)}$. The effects of knockouts on other aspects of the phenotype, however, depend on the pleiotropic effects of the two loci. For example, if $\beta_{(1)X}$ and $\beta_{(1)Y}$ have opposite signs (one is negative and the other is positive), then knockouts of the two loci will have qualitatively similar phenotypic effects along the axis of $\phi_{(1)}$. Thus, it is possible for the phenotypes associated with knockouts of two oppositely imprinted genes to share certain effects.

Conclusion

The analysis presented here demonstrates how the combination of conflict and pleiotropy can drive large phenotypic deviations, both in the phenotype value that is the subject of the intragenomic conflict, and in other phenotype dimensions where both maternally and paternally derived genes share the same optimal value. The model is purely deterministic, and the reduction in fitness is the direct result of natural selection. The detrimental effects of conflict are generic in the model. The fitness at the ESS is always reduced relative to the conflict-free case, excepting very specific combinations of parameter values.

The magnitude of the fitness cost depends both on the magnitude of the conflict, and on the nature of the pleiotropic constraints affecting the loci involved. Phenotypic deviations are linear in the magnitude of the conflict between maternally and paternally derived alleles. Fitness effects scale as the square of the magnitude of this conflict.

The dependence on the nature of the pleiotropic constraints is potentially much more significant, exhibiting a strong nonlinearity. Phenotypic deviations are largest when the pleiotropic effects of the two antagonistic loci are similar. These results suggest that while intragenomic conflict will generically produce maladaptive outcomes, the magnitude of the conflict may not be the most important determinant of how maladaptive those outcomes are. Rather, the mechanisms of action of the loci involved in the conflict primarily determine the magnitude of the fitness cost associated with the ESS.

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