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The evolution of genomic imprinting: Abortion and overshoot explain aberrations

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ABSTRACT

In a previous paper, we presented a quantitative genetic model of the evolution of genomic imprinting in mammals – differential gene expression of an embryonic growth factor gene depending on the parental origin. We have shown that the verbal argument of the genetic conflict hypothesis can be justified by a formal genetic model. The model predicts the evolution of an expressed paternal allele and a silent maternal allele for a growth-enhancing gene increasing maternal resource acquisition, while the reverse pattern will evolve for an inhibitor gene. This may, however, be prevented if there are recessive deleterious mutations on coding regions in the population. In this paper, we examine potential problems for the genetic conflict hypothesis and discuss how the theory can account for them. First, we show that the reverse pattern of genomic imprinting of a growth factor gene can evolve if the risk of abortion in early gestation is enhanced by the overproduction of the growth enhancer. Such a pattern is observed in *Mash2*. Second, paternal disomies (double dose of paternal origin and none from maternal origin) with respect to a part of a chromosome sometimes result in a decrease (rather than an increase) in embryo size. Here we show that this can be explained if the imprinted genes regulate the allocation between placenta and embryo proper by modifying the developmental fate of cells. Third, an alternative non-conflict hypothesis is studied in the same modelling framework, which states that genomic imprinting has evolved because it reduces the risk of spontaneous development of parthenogenetic embryos that cause serious risk to the mother's life (ovarian time-bomb hypothesis). Finally, we point out that genes on the X chromosome receive selection different from autosomes, resulting in the evolution of genomic imprinting in the opposite direction.

Keywords: abortion, genetic conflict, genomic imprinting, *Mash2*, multivariate quantitative genetics, overshoot, parthenogenesis prevention, uniparental disomy.

INTRODUCTION

Recently, genomic imprinting of embryonic genes in mammals has been studied intensively. For these genes, dependent on the parental origin (whether it comes from egg or from sperm), only one allele is actively expressed in the embryo and the other allele is inactive (Peterson and Sapienza, 1993; Gold and Pedersen, 1994; Barlow, 1995; Ohlsson *et al.*, 1995). Some imprinted genes code for an embryonic growth factor or its inhibitor; others

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play important roles in development, and still others are related to behavioural abnormalities after birth.

Genomic imprinting in mammals was noted from the growth retardation and death of embryos of uniparental disomies, which have the maternal duplication and paternal deficiency (or paternal duplication and maternal deficiency) of the whole genome, or of a single chromosome (Solter, 1988). Ferguson-Smith *et al.* (1991) incorporated cells with paternal duplication of the distal chromosome 7 into chimeras and found considerably enhanced growth of the embryos. In contrast, in embryos with maternal duplication of the distal chromosome 7, both maternal alleles of the *Igf2* (insulin-like growth factor type-2) gene were repressed. DeChiara *et al.* (1991) demonstrated that, using gene targeting, the paternally inherited disrupted *Igf2* gene caused growth deficiency but the maternally inherited disrupted gene did not. Nuclease protection and *in situ* hybridization analyses have confirmed that the paternal allele is expressed but the maternal allele is silent in many tissues. Similarly, only the paternally derived alleles are expressed for several genes on human chromosome 15, which are related to behavioural abnormalities, such as Prader-Willi syndrome and Angelman syndrome (Ozcelik *et al.*, 1992; Sutcliffe *et al.*, 1994; Wevrick *et al.*, 1994).

On the other hand, there are genes in which only the maternally derived copy is actively expressed. For example, *Igf2r* (insulin-like growth factor type-2 receptor) is an inhibitor of *Igf2* (Lau *et al.*, 1994), and is expressed only from the maternal chromosome in mice (Barlow *et al.*, 1991). In the mouse, the *H19* gene is imprinted, with the active copy derived from the mother (Bartolomei *et al.*, 1991). *H19* RNA has tumour-suppressor activity (Hao *et al.*, 1993). Some mouse genes controlling the cell cycle are also imprinted: an inhibitor of the Cyclin/CDK complex, $p57^{KIP2}$, is transcribed only from the maternal allele (Hatada and Mukai, 1995), but an activator gene, CDC25, is transcribed only from the paternal allele in some tissues (Plass *et al.*, 1996).

David Haig and his colleagues proposed a genetic conflict hypothesis to explain genomic imprinting as an outcome of evolution based on natural selection (Haig and Westoby, 1989, 1991; Haig and Graham, 1991; Moore and Haig, 1991). Since a female has some possibility of accepting more than one mate throughout her life, the maternal allele of the same gene is more likely to be shared by other embryos of the same mother than the paternal allele. Acquisition of more maternal resource reduces the number, growth or survivorship of the sibs. As a result, the level of maternal resource supply that is optimal for the maternal allele is lower than that optimal for the paternal allele (Haig, 1992). This explains why imprinted genes are likely to be involved in controlling the amount of resources supplied by the mother, and why the paternal alleles are often active and the maternal alleles inactive for embryonic growth factor genes or for genes controlling the post-natal activity attracting attention of the mother. It also explains why the maternal allele tends to be active and the paternal allele tends to be inactive for genes coding for inhibitors of growth factors or genes reducing the demand for resources in general.

In a previous paper, we developed a quantitative genetic model for the evolution of genomic imprinting based on the genetic conflict hypothesis (Mochizuki *et al.*, 1996). We considered the evolution of a *cis*-regulatory region which controls the imprinted expression patterns for a growth factor gene that affects the amount of nutrients obtained from the mother. Assuming that the 'primary imprinting signal' for the parental origin is given, the model predicts that the genes controlling maternal resource supply should evolve to an extreme asymmetry in gene expression between maternally and paternally derived alleles, if

there is some probability of the female accepting multiple mates. Thus the verbal argument of the genetic conflict hypothesis has been justified.

On the other hand, some genes affecting the development of the embryo are not imprinted (e.g. *Igf1*), and some genes are imprinted in mice but not in humans (e.g. *Igf2r*; Kalscheuer *et al.*, 1993; Ogawa *et al.*, 1993). To explain this, we discussed several modified versions of the model, incorporating processes that favour the absence of genomic imprinting. In particular, we studied the possibility that recessive deleterious mutations in the coding region of the gene are accumulated at a low frequency in the population and prevent the evolution of genomic imprinting.

In the present paper, we examine several cases that are apparently inconsistent with the genetic conflict hypothesis. First, there is a mouse gene called *Mash2* that shows imprinting in the opposite direction predicted by the basic model. Since *Mash2* is required for trophoblast development, the basic model would expect an active paternal allele and an inactive maternal allele. However, in reality, the paternal allele becomes repressed by 8.5 days post-coitum, leaving only the maternal allele active (Guillemot *et al.*, 1995). In the following, we show that such a 'reverse' imprinting pattern is in fact the expected evolutionary outcome for a growth-enhancing gene, provided that the risk of abortion in early gestation increases with the gene expression.

Second, paternal uniparental disomies sometimes produce embryos smaller than normal (e.g. Cattanach and Beechey, 1990; Oakey *et al.*, 1995; Hurst and McVean, 1997). This is also apparently inconsistent with the genetic conflict hypothesis, which predicts that paternal disomies are larger than normal embryos (e.g. Haig and Graham, 1991). We show that this is also consistent with the genetic conflict hypothesis if the imprinted gene controls the allocation between embryo and placenta, by modifying resource flows or the developmental fate of cells. Paternal disomy is likely to result in an extreme allocation that 'overshoots' the value for maximum embryo size, causing an embryo smaller than normal.

In addition to the genetic conflict hypothesis, several other explanations have been proposed for genomic imprinting in evolutionary terms (reviewed by Haig and Trivers, 1995; Hurst, 1997). For example, Varmuza and Mann (1994) proposed that imprinting might be a device for protecting female mammals from the potential ravages of ovarian trophoblast disease. Parthenogenetic activation of eggs in the ovary may lead to ovarian tumours, which are quite common in humans and other mammals (Mann and Varmuza, 1994), and then turn an otherwise benign dermoid cyst into a lethal choriocarcinoma. This danger is reduced if the genes necessary for trophoblast growth are inactivated in the ovary of parthenogenetic embryos. We show that the same quantitative genetic model is able to handle this non-conflict hypothesis.

QUANTITATIVE GENETIC MODEL

We first present the quantitative genetic model of evolution, which provides the basis for all the arguments below. We consider an autosomal gene coding for an embryonic growth factor that increases the supply of maternal resources obtained through the placenta, and we discuss the evolution of the *cis*-controlling regulating element of the gene.

The primary imprinting mechanism is still under investigation, but differential methylation during gametogenesis is thought to play an important role (Chaillet *et al.*, 1991; Sasaki *et al.*, 1991, 1993; Ueda *et al.*, 1992; Li *et al.*, 1993; Kelsey and Reik, 1997). Here we simply assume that there are some mechanisms that provide a reliable primary signal to

each allele on its parental origin. We assume that the expression level can be switched between two different levels depending on its parental origin. The state of a *cis*-controlling region is represented by a pair of real numbers (x,y), in which x and y indicate the amount of a growth factor produced by the paternal allele and by the maternal allele, respectively. These are either positive or zero, because they indicate gene expression levels. Their sum, $x + y$, is the total growth factor production per cell. We examine the evolutionary change in x and y, in particular the potential evolution towards asymmetric expression ($\bar{x} \gg \bar{y}$ or $\bar{y} \gg \bar{x}$) from an initial symmetry ($\bar{x} = \bar{y}$). Here, we treat the physiological response of the mother to the growth factor as given.

In a single fertilized egg, there are two alleles (paternal and maternal), each of which has a pair of values (x,y). Let (x_p, y_p) be the paternally derived allele and (x_m, y_m) be the maternally derived allele. By definition, the former produces x_p and the latter produces y_m, and hence the embryo produces $x_p + y_m$ growth factor in total.

The survivorship of the embryo increases with maternal resource supply. It is a function of $x + y$, and is denoted by $W(x + y)$ (see figure 1 in Mochizuki *et al.*, 1996). It is zero (or very small) below a certain threshold, then increases quickly with $x + y$, and finally saturates at a high level. There may also be a peak beyond which it may decline with $x + y$ owing to harmful effects of producing too much growth factor.

The level of embryonic growth factor production $x + y$ that maximizes the survivorship $W(x + y)$ is, however, not optimal, because the embryo shares the common pool of maternal resources with its sibs, either simultaneously or over the mother's reproductive lifetime. Hence reducing the demand for resources will increase the number and the survivorship of sibs that are genetically related to the embryo. The level of growth factor production favoured by natural selection should therefore be lower than the level that achieves the maximum survivorship of the embryo. The optimal growth factor production for the maternally derived gene is still lower than that for the paternally derived gene, because the maternal allele is more likely to have a copy in sibs than the paternal allele if the mother has accepted multiple males. This difference creates a conflict of interest between the alleles within the same cell.

Now we consider the fitness of the (x,y)-allele, defined as the expected number of copies in the following generation for each gene (the multiplication rate from the adult stage to the next adult stage). It also depends on the sex of the gene carrier, and on the population mean traits $(\bar{x},\bar{y})$. Let $\varphi_m(x,y,\bar{x},\bar{y})$ be the fitness for an (x,y)-allele in a male. Similarly, let $\varphi_f(x,y,\bar{x},\bar{y})$ be the fitness of an (x,y)-allele in a female. We call φ_m and φ_f the male and female fitness, respectively.

In the initial population, the two values may be equal ($\bar{x} = \bar{y}$), indicating the absence of genomic imprinting. These change with time owing to natural selection working on the regulatory region. If the heritable values of x and y of individuals in the population are sharply concentrated around their means ($\bar{x}$ and $\bar{y}$), we can calculate one-generation changes in the population averages of two quantitative traits by the product of a genetic variance matrix and a selection gradient vector (see Iwasa *et al.*, 1991):

$$\begin{pmatrix} \Delta\bar{x} \\ \Delta\bar{y} \end{pmatrix} = \begin{pmatrix} G_x & B \\ B & G_y \end{pmatrix} \begin{pmatrix} \beta_x \\ \beta_y \end{pmatrix} \tag{1a}$$

where G_x and G_y are additive genetic variances for x and y, respectively, and B is the additive genetic covariance between them. These are determined by different forces, including

mutation, stabilizing selection, pleiotropy and assortative mating, but here we simply treat them as constant, and assume that the genetic variance–covariance matrix is not degenerate. β_x and β_y are selection gradients with respect to the expression of maternally derived and paternally derived genes in the embryo:

$$\beta_x = \frac{\partial}{\partial x}\frac{1}{2}(\ln\varphi_{\mathrm{m}} + \ln\varphi_{\mathrm{f}}) \text{ and } \beta_y = \frac{\partial}{\partial y}\frac{1}{2}(\ln\varphi_{\mathrm{m}} + \ln\varphi_{\mathrm{f}}) \tag{1b}$$

where the partial derivatives are estimated at the population averages ($(x,y) \to (\bar{x},\bar{y})$). These indicate the direction and magnitude of natural selection working on x and y, respectively. The factor 1/2 indicates sex-limitation; that is, the allele is a paternally derived gene in half of all the generations and a maternally derived gene in the other half. Equations (1a,b) were derived by Iwasa *et al.* (1991) based on the assumption of weak selection, and have been used in computing evolutionary equilibria and evolutionary limit cycles for male sexual ornaments and female mating preferences (e.g. Pomiankowski and Iwasa, 1993; Iwasa and Pomiankowski, 1995).

To specify the dynamics in equation (1), we need to compute the fitnesses $\varphi_{\mathrm{m}}(x,y,\bar{x},\bar{y})$ and $\varphi_{\mathrm{f}}(x,y,\bar{x},\bar{y})$. In this step, we must specify how embryos of the same mother compete for maternal resources and how they are related genetically to each other. We have considered two models of maternal care. In the resource division model, there are two assumptions. First, a reproductive female has a limited amount of resources T and hence the total number of offspring is T divided by the average resource demand per embryo. Second, a $1 - g$ female mates with one male only throughout her life, whereas g females mate with two males equally. Based on these assumptions, we can calculate the fitness of an allele (x,y) in a population.

Alternatively, we consider the sequential care model, in which the mother takes care of offspring one at a time and their father might change with some probability, which gives qualitatively the same predictions as the resource division model (Y. Iwasa, A. Mochizuki and Y. Takeda, unpublished).

Let $R(x + y)$ be the resource acquired from the mother by an embryo. As explained in the Appendix, the female fitness function and male fitness function are, respectively:

$$\varphi_{\mathrm{f}} = \frac{T}{\frac{1}{2}(R(\bar{x} + y) + R(\bar{x} + \bar{y}))}\frac{1}{2}W(\bar{x} + y) \tag{2a}$$

$$\varphi_{\mathrm{m}} = MT\left[\frac{\frac{1-g}{1+g}\frac{1}{2}}{\frac{1}{2}(R(x + \bar{y}) + R(\bar{x} + \bar{y}))} + \frac{\frac{2g}{1+g}\frac{1}{4}}{\frac{1}{4}R(x + \bar{y}) + \frac{3}{4}R(\bar{x} + \bar{y})}\right]W(x + \bar{y}) \tag{2b}$$

Female fitness function in equation (2a) is independent of the polygamy rate g.The first factor in equation (2a) indicates the average number of offspring produced by the mother, which is the total resource T divided by the average resource demand per embryo. The second factor (1/2) is the probability of the allele (x,y) existing in a randomly chosen embryo. The last factor in equation (2a) is the survivorship.

Male fitness in equation (2b) includes the degree of female polygamy g. M is the expected number of times a male is accepted as a mate. The two terms in the braces of equation (2b) give the average of the number of offspring multiplied by the relatedness (see Appendix for details).

The selection gradients for the two quantitative traits are:

$$\beta_x = \frac{1}{2}\frac{\partial}{\partial x}\ln \varphi_m = \frac{1}{2}\left\{-\bar{r}\frac{R'(\bar{x}+\bar{y})}{R(\bar{x}+\bar{y})} + \frac{W'(\bar{x}+\bar{y})}{W(\bar{x}+\bar{y})}\right\} \tag{3a}$$

$$\beta_y = \frac{1}{2}\frac{\partial}{\partial y}\ln \varphi_f = \frac{1}{2}\left\{-\frac{1}{2}\frac{R'(\bar{x}+\bar{y})}{R(\bar{x}+\bar{y})} + \frac{W'(\bar{x}+\bar{y})}{W(\bar{x}+\bar{y})}\right\} \tag{3b}$$

where the prime indicates the derivative with respect to the growth factor level. $\bar{r}$ is the probability for a paternal allele and a randomly chosen allele in a sibling is identical by descent and is given by $\bar{r} = (2 - g)/4$, the relatedness of embryos (see Appendix).

If $R(z) = az$, the result of the evolutionary dynamics is very simple (Mochizuki *et al.*, 1996). If there is some probability of a female accepting multiple males ($g > 0$), the level of growth factor production optimal for the paternal allele (where equation (3a) is zero) is larger than the level optimal for the maternal allele (where equation (3b) equals zero). The evolution of quantitative traits ($\bar{x}$,$\bar{y}$) is always towards an extreme asymmetry of gene expression: in the final population, the maternal allele is not expressed ($\bar{y} = 0$) and all the growth factor is produced by the paternal allele ($\bar{x} > 0$). In the case of a gene coding for an inhibitor that reduces the maternal resource supply, the same theory predicts that evolution creates the opposite expression pattern: only the maternal allele is expressed ($\bar{y} > 0$) and the paternal allele is silent ($\bar{x} = 0$).

EVOLUTION OF REVERSE IMPRINTING

Although the prediction of the basic model explained above is consistent with most results of experimental studies of imprinted genes, there is an exception. *Mash2* is a gene that is required for normal placental development (Guillemot *et al.*, 1994), but the paternal allele is inactivated and the maternal allele is expressed actively (Guillemot *et al.*, 1995). This is the opposite to the prediction of the genetic conflict hypothesis (Haig and Graham, 1991; Mochizuki *et al.*, 1996). This example apparently questions the validity of the genetic conflict hypothesis.

In this section, we explain that this reverse pattern of genomic imprinting is in fact the one predicted by the evolutionary model in equations (1a) and (3), if we consider an additional factor – a risk of abortion occurring in early gestation. A considerable number of conceptions are aborted, most of which are lost in the very early stages of pregnancy around implantation both in humans (Wilcox *et al.*, 1988; Goshen *et al.*, 1994) and in other mammals (Cross *et al.*, 1994). Suppose that a high expression of a gene tends to lead to failed development of the embryo in early gestation, but those embryos that survive the critical stage can grow faster and enjoy a higher survivorship afterwards. If the abortion occurs before substantial resources are invested, the mother can simply replace the embryo by another without losing much. Such developmental failure is more serious for the paternal allele than the maternal allele, because the maternal allele is more likely to have a copy in the replacement sibling embryo. In the extreme case, the risk of developmental failure selects for a reduced level of the growth-enhancing gene on the paternal allele, a level that is even lower than that which is optimal for the maternal allele.

To model the risk of abortion, we assume that the rate of developmental failure increases with the level of gene expression, z. We assume that the probability of an embryo surviving

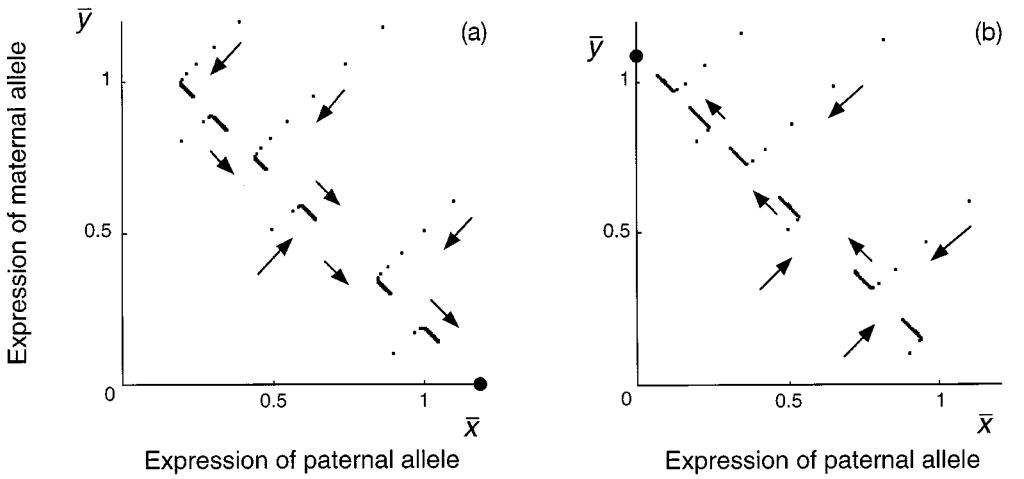

Fig. 1. Evolutionary trajectories of the model with a dose-dependent risk of abortion in early gestation. The two axes are the population averages of paternal allele expression $\bar{x}$ and maternal allele expression $\bar{y}$. (a) Female polygamy rate is $g = 0.5$ and abortion risk is $b = 0.7$. Other parameters are $G_x = G_y = 0.2$, $B = 0$, and survivorship, $W(z)$, is given by $W(z) = (z - 0.5)/(1 + 0.5z^2)$ for $z \geq 0.5$, but is zero for $z < 0.5$ (as illustrated in figure 1 of Mochizuki *et al.*, 1996). Survivorship peaks when $z = 2$. The dynamics are independent of T, A or M. The location of $(\bar{x},\bar{y})$ for every other generation is indicated. Genomic imprinting should evolve and at evolutionary equilibrium only the paternal copy of the gene is expressed. (b) The abortion risk is higher ($b = 1.1$). Other parameters are the same as in (a). The evolutionary outcome is a reverse pattern of imprinting, in which the maternal allele is expressed and the paternal allele is inactive, although the gene codes for a growth factor.

the critical period of abortion is an exponential function, $\exp[-bz]$, where b is the risk coefficient of dose-dependent abortion. The evolution of this situation can be analysed by equations (1a) and (3) if we replace resource acquisition $R(z)$ and survivorship of the embryo $W(z)$ with the following:

$$R(z) \rightarrow az \exp[-bz] \tag{4a}$$

$$W(z) \rightarrow W(z) \exp[-bz] \tag{4b}$$

Equation (4a) gives the resource consumption per embryo in which both aborted and non-aborted embryos are included. Abortion simply reduces the average resource investment per embryo in equation (4a). The survivorship of the embryo is also multiplied by the same factor as shown in equation (4b). If $b = 0$, we have the same model as in Mochizuki *et al.* (1996).

If abortion risk b is positive but small, the level of gene expression optimal for the paternally inherited allele is still larger than that which is optimal for the maternally inherited allele. The evolutionary dynamics in equation (1) then produces a population with paternal allele expressed and maternal allele silent, as illustrated in Fig. 1a. If abortion risk is sufficiently high, this is reversed. Let z_p and z_m be the levels of gene expression optimal for the paternal allele and for the maternal allele, respectively. The condition for reversal is given as follows (see Appendix):

$$\text{if } W'(1/b) > bW(1/b), \text{ then } z_p > z_m \tag{5a}$$

$$\text{if } W'(1/b) < bW(1/b), \text{ then } z_p < z_m \tag{5b}$$

If equation (5b) holds, we find maternal allele active and paternal allele inactive (reverse pattern), as illustrated in Fig. 1b, where the magnitude of dose-dependent abortion b is about 60% higher than in Fig. 1a. If a high level of *Mash2* can cause the abortion of the embryo, equation (5b) may explain the observed pattern (maternal active and paternal inactive) as an evolutionary outcome of genetic conflict, as in Fig. 1b. Equation (5) also tells us that these two situations are distinguished only by the intensity of dose-dependent abortion b and the shape of the embryonic survivorship function $W(z)$, but is independent of female polygamy rate g and fecundity T/a.

The reverse (i.e. $z_p < z_m$) is possible only when the abortion occurs early in gestation before the mother invests a lot in the embryo. If the abortion occurs after the mother has finished investing in the embryo, the reverse does not occur. This is shown by the case with equation (4b) and $R(z) = az$, which always results in $z_p > z_m$.

WHY PATERNAL DISOMIES ARE SOMETIMES SMALLER

The genetic conflict hypothesis predicts that any gene of paternal origin should act to enhance the supply of maternal resources and the growth of the embryo (compared to the corresponding gene of maternal origin). Hence we expect enhanced growth of a paternal disomy (an embryo with a double dose of paternal origin) compared with a normal embryo (with one copy of each). The cases of *Igf2* and *Igf2r* fit this prediction, and have been considered as major support for the genetic conflict hypothesis (Haig and Graham, 1991; Haig and Trivers, 1995). However, there are some cases in which paternal disomy is associated with smaller embryos than normal, for example distal chromosome 17 (Cattanach and Beechey, 1990) and distal chromosome 18 (Oakey *et al.*, 1995; see Hurst and McVean, 1997). These are apparently in conflict with the basic model.

Experimental results suggest that genomic imprinting has a strong effect on the proportions of placenta and embryo proper, rather than the absolute size of the embryo. Placental development often depends on paternally expressed genes and embryonic growth on maternally expressed genes (Barlow, 1995). Parthenogenetic and gynogenetic embryos, whose genomes are entirely maternally derived, have a poorly developed trophoblast but a relatively normal embryo proper (McGrath and Solter, 1984; Surani *et al.*, 1986; Nagy *et al.*, 1987; Clarke *et al.*, 1988; Thomson and Solter, 1988; Solter, 1988). In contrast, androgenetic embryos, with an entirely paternally derived genome, have well-developed extra-embryonic tissues, particularly those that are trophoblast-derived, but limited embryonic development (Surani *et al.*, 1986; Solter, 1988; Bartolomei *et al.*, 1993; Fundele and Surani, 1994). In chimeras of androgenetic cells (with double copies of paternal origin) and cells from a normal embryo, most androgenetic cells go to the placenta and they are not detected in newborn mice (Solter, 1988). In contrast, in chimeras with maternal disomy and normal embryos, cells from maternal disomy tend to remain in the embryo (inner cell mass) and not in the placenta.

This implies that imprinted genes control the developmental fate of cells. Since the placenta is the machinery to acquire resources from the mother's body, a large placenta is considered an indication of the aggressiveness of the embryo, and the relatively large size of the placenta in paternal disomies fits the genetic conflict hypothesis. However, too much

emphasis on placental development may result in a malfunction of the whole embryonic system, which may explain the smaller embryo in some paternal uniparental disomies.

To express this idea, we consider a gene controlling the allocation of maternal resources between placenta and embryo. It may be a gene affecting the developmental fate of cells. We denote the fraction of resources allocated to the placenta by u ($0 \le u \le 1$). The remaining fraction, $1 - u$, is allocated to the embryo. Suppose that the allocation is controlled by the paternal and maternal alleles, and that allocation increases with the total gene product: $u = z/(m + z)$, where $z = x + y$ and m is a constant corresponding to the growth factor level realizing $u = 0.5$ (placenta and embryo proper are of the same size). Since gene expression is positive or zero ($z \ge 0$), the ratio is between 0 and 1.

Let E be the size of the embryo and P be the size of the placenta. Since maternal resources are allocated to the growth of embryo and placenta, the total amount of resource acquisition from the mother is proportional to their sum, $E + P$. To achieve a high rate of maternal resource supply, both placenta and embryo proper are required to be large; and hence, if we plot total resource acquisition as a function of allocation fraction u, it should be a humped curve with a peak for an intermediate value. In the example illustrated in Fig. 2, embryo size $E(u)$, placenta size $P(u)$ and the total resource acquisition (or maternal expenditure) $E(u) + P(u)$ all have humped curves as functions of allocation fraction u, but their peaks are at different values: embryo size $E(u)$ has a peak at a small u, which we denote by u_e; placenta size $P(u)$ has its maximum at a larger u; and the total resource acquisition from the mother has a peak in between these two. We simply assume that the fitness of an embryo is proportional to embryo size.

The optimal allocation fractions for the embryonic alleles (paternal and maternal) are smaller than u_e, the allocation fraction resulting in the largest embryo size (see Fig. 2), because the total resource acquisition $E(u) + P(u)$ has a peak larger than u_e, and because sequestering resources from the mother would cause a reduction in the expected number of siblings or their size. However, the need to avoid higher maternal resource consumption (thus reducing sibling fitness) is stronger for the maternal allele than for the paternal allele. As a consequence, the optimal for the maternal allele, the optimal for the paternal allele and the optimal for embryo size would satisfy: $u_{mat} < u_{pat} < u_e$.

We can apply the evolutionary dynamics in equation (1) to this model, simply by replacing the quantities as follows:

$$W(z) \to E(\hat{u}(z)) \tag{6a}$$

$$R(z) \to E(\hat{u}(z)) + P(\hat{u}(z)) \tag{6b}$$

where $\hat{u}(z) = z/(m + z)$. Equations (6a) and (6b) indicate that the fitness of an embryo is proportional to size E, and that the total supply of maternal resources is proportional to the sum $E + P$.

As a consequence of natural selection working on the paternal and maternal alleles in the same embryo, an extreme asymmetry of gene expression should evolve, with the maternal allele expressing the minimum possible value ($\bar{y} = 0$) and the paternal allele expressing its own optimum level ($\bar{x}$ satisfying $\bar{x}/(m + \bar{x}) = u_{pat}$). The allocation fraction in the final population is the same as the paternal optimum (u_{pat}), which is smaller than u_e (Figs 2a, 2b). As illustrated in Fig. 2, the optimum for the paternal allele is that which realizes an embryo size larger than the optimum for the maternal allele but smaller than the optimum for maximum embryo size ($E(u_{mat}) < E(u_{pat}) < E(u_e)$).

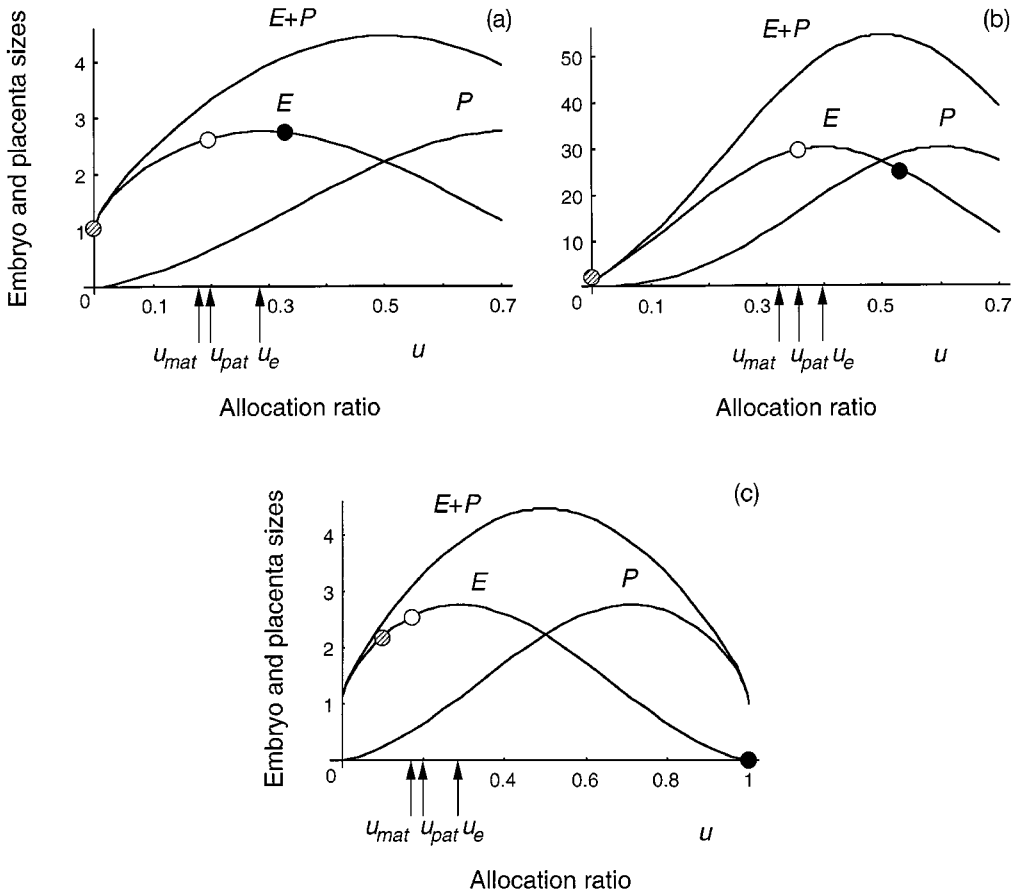

Fig. 2. Embryo size, E, placenta size, P, and the total maternal expenditure, $E + P$, for different fractions u of the maternal resources allocated to the growth of the placenta. The embryo sizes of paternal disomy, normal embryo and maternal disomy are indicated by a solid circle, an open circle and a hatched circle, respectively. Three arrows indicate the allocation fractions realizing the optimum for the maternal allele (u_{mat}), the optimum for the paternal allele (u_{pat}) and the one maximizing embryo size (u_e). E and P are $E = C(1-u)\exp[\sqrt{u(1-u)}K]$ and $P = Cu\exp[\sqrt{u(1-u)}K]$, where C and K are constants. These are derived from the following considerations: The rate of supply of maternal resources is proportional to their geometric mean, $\sqrt{EP}$. The maternal resources are allocated to the placenta and embryo proper with ratio $u : 1-u$, and their sizes grow as: $(dP)/(dt) = us\sqrt{EP}$, $(dE)/(dt) = (1-u)s\sqrt{EP}$, where s is a growth rate. Then, the sizes of E and P after a certain period of time may be given by the above, if K is the period of time multiplied by s. (a) Parameters are: $K = 3$, $g = 0.2$, $C = 1$, $m = 1$. The ratio is $u = z/(m+z)$, with $m = 1$. Paternal disomy (solid circle) is larger than the normal embryo (open circle). (b) Different parameters: $K = 8$, $g = 0.8$, $C = 1$, $m = 1$. The paternal disomy has a very enhanced allocation fraction and overshoots the value required for maximum embryo size. The paternal disomy (solid circle) is smaller than the normal embryo (open circle). (c) When enhanced gene expression reduces the allocation to the placenta: $u = m/(m+z)$, with $m = 1$. Other parameters are the same as in (a). The paternal allele is silent and the maternal allele is active at the evolutionary equilibrium. The paternal disomy has a very high allocation fraction ($u = 1$).

A paternal disomy has a double dose of paternal gene, producing a double gene product as in a normal embryo. Let $\bar{x}$ be the gene expression in a normal embryo. A paternal disomy has a gene expression of $2\bar{x}$ and an allocation fraction of $\hat{u}(2\bar{x})$. The allocation fraction would increase but not double, because of the non-linearity of the ratio–dose relationship. In Fig. 2a, the paternal disomy with a double dose of the paternal allele (solid circle) is larger than the normal embryo (open circle). In contrast, in Fig. 2b, overshoot occurs, and the paternal disomy is smaller than the normal embryo ($E(\hat{u}(\bar{x})) > E(\hat{u}(2\bar{x}))$). Whether overshoot occurs or not depends on the shape of the functions $E(u)$ and $P(u)$, and the degree of polygamy g.

Now we consider a gene allocating less to the placenta. To be specific, the allocation fraction to the placenta is a decreasing function of total gene product in the cell: $\hat{u}(z) = m/(m+z)$, where m is constant (z for $u = 0.5$). Then the paternal allele evolves to be silent and the maternal allele evolves to be active. The normal embryo has u_{mat}, which is realized by $\bar{x} = 0$ and $\bar{y}$ satisfying $m/(m+\bar{y}) = u_{\text{mat}}$. The paternal disomy has no gene expression and hence the allocation fraction is very high ($\hat{u}(0) = 1$). Maternal disomy has a double dose of active maternal alleles and realizes a smaller allocation fraction $\hat{u}(2\bar{y})$, as illustrated in Fig. 2c.

Since gene expression is constrained to be positive or zero, the conflict of the paternal and maternal alleles is settled only when one of them becomes inactive. The other then realizes the optimal allocation fraction for itself. If the gene increases its allocation to the placenta, the paternal allele is active and the maternal allele is inactive at equilibrium (Figs 2a,b). If instead the gene reduces its allocation to the embryo, the paternal allele is inactive and the maternal allele is active (Fig. 2c). In either case, the paternal disomy can be larger or the smaller than the normal embryo depending on the shape of the curves $E(u)$ and $P(u)$ and the polygamy rate, g.

OVARIAN TIME-BOMB

Apart from the genetic conflict hypothesis, several other explanations have been proposed for the evolution of genomic imprinting. For example, Varmuza and Mann (1994) suggested that imprinting might have evolved as a device to protect female mammals from the potential ravages of ovarian trophoblast disease, which is caused by the spontaneous start of the development of unfertilized eggs, which are hence time-bombs. If the maternal allele for the growth factor gene is silent ($\bar{y} = 0$), the risk of ovarian trophoblast disease would be reduced, to the advantage of both parents, thus favouring the reduction of maternal gene expression. This ovarian time-bomb hypothesis can work in the absence of a conflict of interest between paternal and maternal alleles, and is regarded the most likely alternative hypothesis by experimental biologists (Gilligan and Solter, 1995; and other chapters in Ohlsson *et al.*, 1995).

It has been suggested that the main problem with the ovarian time-bomb hypothesis is that it can only explain the inactivation of maternal growth factor genes, but not the inactivation of paternal genes (Haig and Trivers, 1995; Hurst, 1997). However, this is only the case if the hypothesis is restricted to growth enhancers; if it also encompasses growth inhibitors, then we can expect that these will be maternally active but paternally inactive. The ovarian time-bomb hypothesis, therefore, may explain the inactivity of paternal genes.

The modelling of the hypothesis can be done simply by considering an additional factor of female mortality caused by parthenogenetic embryos. For clarity of argument, we

examine the case in which there is no conflict between two alleles – the female accepts only a single male and all the offspring produced by a mother have a common father. There is a small probability that unfertilized eggs start the parthenogenetic development that results in a cancer killing the female. However, this is possible only when the parthenogenetic embryo can receive sufficient maternal resources. We assume that the growth of a parthenogenetic embryo increases linearly with the growth factor in the embryo, $y + \bar{y}$. Here the parthenogenetic embryo is assumed to have the same genome as the mother (apomixis). The fitness of the allele (x,y) in a female and a male is, respectively:

$$\varphi_{\mathrm{f}} = \frac{T}{a\left(\dfrac{y+\bar{y}}{2} + \bar{x}\right)} \frac{1}{2} W(y + \bar{x}) \exp[-c(y + \bar{y})] \tag{7a}$$

$$\varphi_{\mathrm{m}} = \frac{T}{a\left(\dfrac{x+\bar{x}}{2} + \bar{y}\right)} \frac{1}{2} W(x + \bar{y}) \exp[-2c\bar{y}] \tag{7b}$$

In the absence of the risk of parthenogenetic development ($c = 0$), equations (7a) and (7b) are the same if x and y are exchanged. Then there is no systematic selective force to favour imprinting. If there is a risk of parthenogenetic development ($c > 0$), however, there is a pressure to make $\bar{y}$ smaller. In addition, there is stabilizing selection in normal embryos working to achieve some intermediate levels of total gene expression, which tries to keep their sum $\bar{x} + \bar{y}$ near an intermediate value, thus making paternal allele expression $\bar{x}$ larger to compensate for the reduction in $\bar{y}$.

The selection gradient is calculated as:

$$\beta_x = \frac{1}{2} \frac{\partial}{\partial x} \ln \varphi_{\mathrm{m}} = \frac{1}{2} \left\{ -\frac{1}{2} \frac{1}{\bar{x} + \bar{y}} + \frac{W'(\bar{x} + \bar{y})}{W(\bar{x} + \bar{y})} \right\} \tag{8a}$$

$$\beta_y = \frac{1}{2} \frac{\partial}{\partial y} \ln \varphi_{\mathrm{f}} = \frac{1}{2} \left\{ -\frac{1}{2} \frac{1}{\bar{x} + \bar{y}} + \frac{W'(\bar{x} + \bar{y})}{W(\bar{x} + \bar{y})} - c \right\} \tag{8b}$$

Figure 3a illustrates the evolutionary trajectory of equation (1). At equilibrium, the maternal allele is silent ($\bar{y} = 0$) and the paternal allele produces its optimum that makes equation (8a) zero.

If the gene codes for a growth inhibitor so that the embryo with more gene product has a slower growth rate, the evolutionary dynamics predict the opposite. The amount of maternal resources received by the embryo, the survivorship and the mortality risk of the mother by ovarian cancer are all decreasing functions of growth factor production in the embryo. As shown in Fig. 3b, the gene should then evolve to genomic imprinting with the maternal allele expressed and the paternal allele inactive.

This clearly shows that genomic imprinting can evolve without genetic conflict between the alleles. The direction is the same as for the genetic conflict hypothesis: a growth factor gene with paternal allele expressed and maternal allele silent, and a growth inhibitor gene with maternal allele expressed and paternal allele inactive.

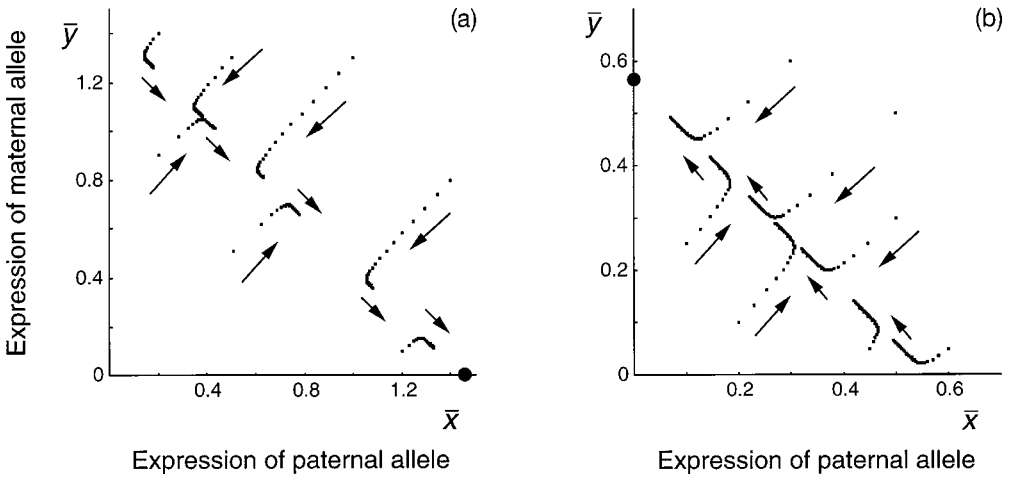

Fig. 3. Evolutionary trajectories of the model with genes affecting the risk of ovarian tumour. Genomic imprinting evolves to reduce the risk of ovarian tumour developing from a parthenogenetic embryo. To compensate for the reduction in maternal allele expression, the paternal allele evolves to be expressed more, resulting in genomic imprinting. Perfect female monogamy is assumed ($g = 0$), implying that there is no conflict between maternal and paternal alleles. The location of $(\bar{x},\bar{y})$ for every other generation is indicated. (a) Growth factor evolution. Equations are given in equations (1) and (8) with parameters: $c = 0.02$, $G_x = G_y = 0.2$, and $B = 0$. The survivorship function is the same as in Fig. 1. (b) Evolution of genomic imprinting for the gene coding for a growth inhibitor. Survivorship of an embryo is given as a function of the amount of the growth-inhibitor product $z = x + y$, $W_i(z) = (F_m - z - 0.5)/(1 + 0.5(F_m - z)^2)$, for $z < F_m - 0.5$, but is zero for $z \geq F_m - 0.5$. $F_m = 2$. The resource allocation to an embryo decreases with z as $a(F_m - z)$. Further explanation is given in appendix C and figure 6A of Mochizuki *et al.* (1996). The female suffers mortality due to ovarian tumour, expressed as an additional survivorship factor of $\exp[-c\{F_m - (y + \bar{y})\}]$. The dynamics are independent of T, a and M. The location of $(\bar{x},\bar{y})$ for every other generation is indicated. There is a globally stable equilibrium on the $\bar{y}$-axis in which the paternal allele is silent. Both figures indicate that an extreme asymmetry of gene expression can evolve without conflict between genes ($g = 0$), and that a growth factor evolves to be paternally expressed (maternally imprinted) and a growth inhibitor to be maternally expressed (paternally imprinted).

DISCUSSION

In this paper, we have studied patterns of genomic imprinting in mammals that apparently are in conflict with the predictions of the genetic conflict hypothesis. We have shown that these observations are consistent with the genetic conflict hypothesis if further processes are considered.

Reverse imprinting pattern and abortion

Mash2 is a gene required for the development of the placenta, an organ necessary for resource acquisition (Guillemot *et al.*, 1994), for which the genetic conflict hypothesis in its simplest form would predict the expression of the paternal allele and the inactivation of the maternal allele. However, *Mash2* is imprinted in the opposite direction – the maternal copy is expressed and the paternal copy is silent (Guillemot *et al.*, 1995). This reverse pattern

can be explained if a higher expression of this gene increases the risk of developmental failure in early gestation. Such a risk is more serious to the paternal allele than to the maternal allele, because the former has a less than 50% chance of being present when the mother replaces it by its sib. For this logic to work, developmental failure should occur in the early stages, before the mother has invested substantial resources. For humans (Wilcox *et al.*, 1988; Goshen *et al.*, 1994) and other mammals (Cross *et al.*, 1994), the rate of abortion during gestation is very high, and most failures occur in the very early stages of pregnancy around implantation. In addition, the risk of abortion needs to increase with the amount of gene expression. If the dose-dependent abortion rate is sufficiently large (see equation 5), the optimal for the paternal allele becomes smaller than the optimal for the maternal allele, and hence evolution creates the reverse imprinting pattern (Fig. 1b).

According to the hypothesis, there are two different kinds of abortion caused by the over- or under-expression of *Mash2*. The abortion in the critical stage of early gestation should be caused by overproduction. In contrast, the abortion that occurs in the later stage is caused by a malfunction of the placenta and a shortage of nutrients supplied by the mother, which is caused by the underproduction of *Mash2*. These two must be distinguished in designing the test of the hypothesis. In addition, we need to estimate the rate of abortion occurring around implantation, but many experiments have examined only those embryos that succeed to implantation.

Smaller-sized paternal disomy

Some paternal disomies in mice are known to be smaller than the normal embryo with both paternal and maternal genes; for example, distal chromosome 17 (Cattanach and Beechey, 1990) and distal chromosome 18 (Oakey *et al.*, 1995; see recent extensive review by Hurst and McVean, 1997). This cannot be accounted for by the genetic conflict hypothesis in its simplest form, which predicts that a double dose of paternal alleles should enhance embryonic growth. However, this prediction is not valid when the gene products of imprinted genes have a non-linear relationship with embryo size.

As a simple mechanism generating non-linearity, we examined a gene controlling the allocation fraction between the placenta and embryo, both of which are needed for rapid growth of the embryo. The genetic conflict hypothesis predicts that maternal and paternal alleles will have different optimal levels of gene expression. The paternal alleles will pull the expression level towards increasing embryo size, while the maternal alleles will pull in the opposite direction. This will lead to the evolution of full imprinting, with the maternal allele inactive and the paternal allele active. Since the relationship between gene expression and embryo size is not monotonic, the double dose of active paternal alleles may overshoot the level required for maximal embryo size, leading to an embryo smaller than normal (Fig. 2b).

From examination of different parameter values, the simple model in Fig. 2 clarifies several points for the genes controlling allocation between the placenta and embryo proper. First, the genes enhancing the size of the placenta relative to the embryo should evolve genomic imprinting with the paternal allele active and the maternal allele silent (Fig. 2b). In contrast, genes reducing the placenta to embryo ratio should evolve to a pattern with the paternal allele inactive and the maternal allele active (Fig. 2c). Second, if the effect of enhanced allocation to the placenta is small, we expect it will always result in a larger embryo than normal. This ought to be seen in chimera experiments using cells from a

paternal disomy mixed with cells of a normal embryo. This avoids the extreme effect of a doubling of gene expression seen in paternal disomy. Third, a paternal disomy may result in a larger or smaller embryo. This depends on the shape of the curve relating embryo size to the allocation fraction and the degree of female polygamy.

Ovarian time-bomb, non-conflict hypothesis

We have demonstrated that the same quantitative genetic model can be used to examine the alternative hypothesis that genomic imprinting has evolved to reduce the risk of spontaneous development of malignant parthenogenetic tumours that are a serious risk to the mother's life (Varmuza and Mann, 1994). This ovarian time-bomb hypothesis is regarded as a persuasive alternative by many experimental biologists (Gilligan and Solter, 1995; and other chapters in Ohlsson *et al.*, 1995). We have shown that genomic imprinting can evolve without any conflict between maternal and paternal alleles (i.e. when a female mates with a single male only).

The ovarian time-bomb hypothesis has been criticized as being unable to explain the evolution of genes which are only maternally expressed, such as *Igf2r* (Haig and Trivers, 1995). However, it is possible to explain paternal imprinting if we assume that the risk of spontaneous development of parthenogenetic embryos can be reduced by a higher expression of growth inhibitor genes. Stabilizing selection works on both alleles to maintain the total gene product near intermediate values in normal embryos, although the exact optimum may differ between paternal and maternal alleles. If the enhanced expression of growth inhibitor is favoured in the maternal allele to reduce the risk of parthenogenetic development, then the level is compensated for by the reduced expression of the paternal allele, resulting in imprinting with the maternal allele expressed but the paternal allele silent. Thus the time-bomb hypothesis can explain the inactivity of the paternal allele of growth inhibitor genes without resorting to the 'innocent bystander' explanation (Varmuza and Mann, 1994).

The second criticism of the ovarian time-bomb hypothesis is that, if imprinting of one gene can effectively shut down the possibility of parthenogenetic development, there is no need for so many imprinted genes. However, if imprinting of one gene can reduce the risk of malignant ovarian cancer but cannot eradicate it, then other genes can also evolve imprinting by the same mechanism.

Genomic imprinting of genes on the X chromosome

The arguments so far are for autosomal genes. For the genomic imprinting of genes on the X chromosome, some observed patterns do not match with the predictions of the genetic conflict hypothesis, and more importantly there is an additional selection process at work that leads to genomic imprinting in the direction opposite to that predicted by the genomic conflict hypothesis (Iwasa and Pomiankowski, in press).

For example, Thornhill and Burgoyne (1993) have reported that the size of the embryo of female XO mice that have inherited a single X chromosome is different if the X comes from the mother (Xm) or from the father (Xp). The genomic conflict hypothesis predicts that an XpO individual with paternal X should be larger in size than an XmO individual with maternal X, because of the lower relatedness to other sibs. However, the opposite is observed: XmO is larger than XpO (Thornhill and Burgoyne, 1993).

X-linked genes have a pattern of inheritance different from autosomes. The maternal X is transmitted equally to female and male offspring, but the paternal X is always passed onto female offspring. This inheritance asymmetry pre-adapts X-linked genes to have sex-specific expression controlled by imprinting. If the optimal level of gene expression of an X-linked gene in female embryos is different from that in male embryos, differential gene expression is based on whether it is on a maternally inherited X chromosome or on a paternally inherited X chromosome.

To test this idea, consider the pattern of X-linked imprinting in the mouse which controls fetal size dimorphism. Using an additive model, we assume that maternal Xm codes for fetal size m and paternal Xp is imprinted and codes for fetal size p. Given random X inactivation, female (XmXp) fetuses have size $(m + p)/2$. It is plausible to assume that the optimal size for the male embryo may be larger than the optimal size for the female embryo because males are larger than females in terms of the fetus (Thornhill and Burgoyne, 1993). Then the evolution of additive genetic values m and p converge to the equilibrium at which male size m is larger than female size $(m + p)/2$. This then predicts that the embryo size of female XmO mice expresses m and is the same size as normal males (XmY), whereas XpO females express p, which is smaller than both normal males (XmY) and normal females (XmXp). This is exactly the pattern observed in mice (Thornhill and Burgoyne, 1993).

A similar genomic imprinting of the human X chromosome was reported by Skuse *et al.* (1997), who showed that individuals inheriting a paternal X chromosome (XpO) had superior social skills compared to those inheriting a maternal X chromosome (XmO). This can also be explained if X-linked imprinting has evolved to control sex-specific expression of genes with X-linked inheritance, given there has been selection for different social skills in females and males.

To guarantee a single active X chromosome per cell, there is a counting mechanism, but the X chromosome to be inactivated is always the paternal one in the extra-embryonic tissues of the mouse, realized by the carry-over of the methylation difference made during gametogenesis (Kay *et al.*, 1994; Monk, 1995), and X-linked genes with paternal origin tend to be repressed more often than those with maternal origin before X chromosome inactivation for dosage compensation starts (Latham and Rambhatla, 1995). The interpretation of these patterns requires great care and the consideration of many other aspects, including the role of deleterious mutations in favouring a random X-inactivation in the embryo (Moore and Haig, 1991). A full treatment is beyond the scope of the present paper. However, the patterns of these phenomena seem to be interpreted more readily by the consideration that the X chromosome of different parental origin will experience embryos of different sex, rather than by the differential relatedness to the sibs owing to the mother's polygamy.

CONCLUSION

In the present paper, we have shown that the genetic conflict hypothesis can predict patterns that at first sight look to be inconsistent with the basic model. We suggest that the genetic conflict hypothesis is the most promising hypothesis for the evolution of genomic imprinting of autosomal genes for the following reasons. First, for genomic imprinting to evolve, no special mechanisms of interaction between genes are required, but simply the modification of the *cis*-regulating regions of genes coding for growth factor or growth inhibitor. Second, genetic conflict between the alleles is always present and can be sub-

stantial because, according to behavioural ecological studies using molecular markers, no mammals are perfectly monogamous, and extra-pair copulation is prevalent even among socially monogamous species, both in muroid rodents (Dewsbury, 1984) and humans (Harvey and Harcourt, 1984; Smith, 1984). Third, and probably the most difficult condition to meet, is the availability of information on parental origin of the chromosome. The genetically imprinted mouse genes (*Mash2*, *Ins2*, *Igf2*, *H19*) are clustered into a chromosomal domain (Eden and Cedar, 1995). Similarly, a group of genomically imprinted human genes (*SNRPN*, *PAR-5*, *PAR-1*, *IPW*) are located within a single region of several hundred kb (Ozcelik *et al.*, 1992; Wevrick *et al.*, 1994), and are thought to be controlled by an imprinting-controlling element (Reis *et al.*, 1994; Sutcliffe *et al.*, 1994; Buiting *et al.*, 1995). Such clustering may suggest that information on parental origin may not be readily available, but once it becomes available, genomic imprinting may be relatively easy to evolve.

On the other hand, we should not dismiss alternative processes, because some of them, such as the ovarian time-bomb hypothesis examined in this paper, may promote evolution of genomic imprinting in the same direction as the genetic conflict hypothesis. For genes on X chromosomes, the imprinting is promoted by inheritance asymmetry and sex differences, which work even under perfect female monogamy. These different processes leading to evolution of imprinting are not mutually exclusive, and they may work simultaneously. We need a more careful quantitative estimate of various processes – such as the rate of abortion, its does dependence, the rate of parthenogenesis, the frequency of deleterious mutations in the population – to achieve a better understanding of the evolution of genomic imprinting.

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APPENDIX

The derivation of equations (2a) and (2b) can be made in the same way as in Mochizuki *et al.* (1996). Consider female fitness, the expected number of copies of a gene (x,y) possessed by a reproductive female in the current generation. We need to specify the genetic state of the alternative allele in the same female, denoted by (x',y'), and the genotypes of her mate(s). She accepts a single male with probability $1 - g$. Let (x_1,y_1) and (x_2,y_2) be two alleles at the same locus possessed by the male. The female may accept two males with probability g. The two genes of the first male are (x_1,y_1) and (x_2,y_2); those of the second male are (x_3,y_3) and (x_4,y_4). We then calculate the number of copies of the gene (x,y) in the following generation in a straightforward way. Then we put all the genotypes except for (x,y) to equal the population average values $(\bar{x},\bar{y})$; that is, $(x',y') = (x_1,y_1) = .. = (\bar{x},\bar{y})$. This is because, in calculating the selection gradient, we need to compute the partial derivative of the fitness with respect to x or y at the population mean values $(\bar{x},\bar{y})$. The procedure is the same as in appendix A of Mochizuki *et al.* (1996), except that the average resource acquisition $a(x + y)$ in the denominator is generalized as $R(x + y)$. The result is given by equation (2a).

Male fitness can also be derived in the same way, and the result is given by equation (2b). We calculate the average of two cases: when the female accepts a single male throughout her life (with

probability $1 - g$) and when she accepts two males (with probability g). However, we note that the weights of these two cases used in calculating male fitness are $(1 - g)/(1 + g)$ and $2g/(1 + g)$, instead of $(1 - g)$ and g. The factor of 2 comes from the fact that two males experience the latter mating situation.

We can generalize the results to the case in which a female accepts n males with probability p_n, which sire the offspring of the female equally. After arithmetic similar to the above, we have female fitness φ_f the same as equation (2a) and male fitness φ_m as:

$$\varphi_m = \frac{M}{\sum_{n'=1} n' p_{n'}} \sum_{n=1} np_n \left\{ \frac{T}{\frac{1}{2n} R(x + \bar{y}) + \frac{2n-1}{2n} R(\bar{x} + \bar{y})} \frac{1}{2n} W(x + \bar{y}) \right\} \tag{A1}$$

Note that the weighting used in equation (A1) is np_n instead of p_n, because females accepting many males have more opportunity to contribute to the calculation of equation (A1) than females with fewer matings. With $p_1 = 1 - g$, $p_2 = g$, $p_3 = p_4 = .. = 0$, equation (A1) becomes equation (2b).

The partial derivatives of fitness functions give selection gradients. Direct calculation for the case of equation (A1) gives equation (3) in the text in which $\bar{r}$ is given as:

$$\bar{r} = \sum_n p_n \frac{1}{2n} \tag{A2}$$

which is simply the relatedness of embryos of the same mother. For the case examined in the text ($p_1 = 1 - g$, $p_2 = g$, $p_3 = p_4 = .. = 0$), we have:

$$\bar{r} = (1 - g)\frac{1}{2} + g\frac{1}{4} = \frac{2 - g}{4}$$

The quantity for a female's gene is 1/2 because half of the embryos carry the gene but the other half carry a different allele of the same female. $\bar{r}$ is less than 1/2 because of the possibility of multiple mating of the female. If the female always accepts n males, it is $1/2n$. The $\bar{r}$ value given by equation (A2) is the average of this quantity for different numbers of mates.

Note that $\bar{r} \leq 1/2$, where the equality holds only when the female is perfectly monogamous.

Denote the following function by $f(\bar{z};\bar{r},b)$:

$$f(\bar{z};\bar{r},b) = -\bar{r}\frac{1}{\bar{z}} - (1 - \bar{r})b + (\ln W(\bar{z}))' \tag{A3}$$

Then we have $2\beta_x = f(\bar{z};\bar{r},b)$ and $2\beta_y = f(\bar{z};1/2,b)$. Let z_p and z_m be the optimum growth factor level for the paternal allele and for the maternal allele, respectively. Then

$$0 = f(z_p;\bar{r},b) \text{ and } 0 = f(z_m;1/2,b)$$

The isoclines are given by parallel lines of slope minus 1: $\bar{x} + \bar{y} = z_m$ and $\bar{x} + \bar{y} = z_p$. Depending on whether z_m or z_p is the larger, the evolutionary outcome is paternal inactivity or maternal inactivity. Let $\hat{z}(r,b)$ be the solution of $f(z;r,b) = 0$. We assume that there is stabilizing selection at work in terms of the level of growth factor production, implying that the second derivative of the fitness function has a negative second derivative with respect to growth factor production:

$$\frac{\partial}{\partial z} f(\bar{z};\bar{r},b) = \bar{r}\frac{1}{\bar{z}^2} + (\ln W(\bar{z}))'' < 0 \tag{A4}$$

Calculating the partial derivative of $f(\hat{z}(r,b);r,b) = 0$ with respect to r would lead to

$$\left[\frac{r}{z^2} + (\ln W(z))''\right]\frac{\partial \hat{z}}{\partial r} = \frac{1}{z} - b$$

This implies that:

$$\text{if } z > \frac{1}{b}, \text{ then } \frac{\partial \hat{z}}{\partial r} > 0 \tag{A5a}$$

$$\text{if } z < \frac{1}{b}, \text{ then } \frac{\partial \hat{z}}{\partial r} < 0 \tag{A5b}$$

Second, we consider the sign of function $f(z;r,b)$ with r and b fixed. As z increases, it decreases monotonically and changes sign from positive to negative only once, because of the assumptions of equation (A4). Whether the cross occurs larger or smaller than $1/b$ is determined by the sign of $f(1/b;r,b)$. By taking $f(1/b;r,b) = -b + (\ln W(1/b))'$, we have:

$$\text{if } (\ln W(1/b))' > b, \text{ then } z > \frac{1}{b} \tag{A6a}$$

$$\text{if } (\ln W(1/b))' < b, \text{ then } z < \frac{1}{b} \tag{A6a}$$

Combining equations (A5) and (A6) with $z_p = \hat{z}(\bar{r},b)$, $z_m = \hat{z}(1/2,b)$ and $\bar{r} < 1/2$, we have the conclusion in equation (5) in the text.