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Genetic conflicts in genomic imprinting

Austin Burt[1*] **and Robert Trivers**[2]

[1]*Department of Biology, Imperial College, Silwood Park, Ascot, Berkshire SL5 7PY, UK*
[2]*Anthropology, Rutgers, New Brunswick, NJ 08903-0270, USA*

The expression pattern of genes in mammals and plants can depend upon the parent from which the gene was inherited, evidence for a mechanism of parent-specific genomic imprinting. Kinship considerations are likely to be important in the natural selection of many such genes, because coefficients of relatedness will usually differ between maternally and paternally derived genes. Three classes of gene are likely to be involved in genomic imprinting: the imprinted genes themselves, *trans*-acting genes in the parents, which affect the application of the imprint, and *trans*-acting genes in the offspring, which recognize and affect the expression of the imprint. We show that coefficients of relatedness will typically differ among these three classes, thus engendering conflicts of interest between Imprinter genes, imprinted genes, and imprint-recognition genes, with probable consequences for the evolution of the imprinting machinery.

Keywords: conflicts of interest; genomic imprinting; kinship; parent–offspring conflict; parent-specific gene expression

1. INTRODUCTION

For some genes in mammals and plants, maternally and paternally derived alleles have different patterns of expression (Barlow 1995; Reik & Surani 1997). In the usual case, one allele is silent and the other active; sometimes this difference is seen in some tissues and not in others, and sometimes there is only a quantitative difference in gene expression. This parent-specific gene expression is presumably due to differential imprinting of the alleles in the maternal and paternal germ lines. Why would such a system evolve? One likely explanation is that maternally and paternally derived genes have different coefficients of relatedness to many relatives, and so have different optimal levels of expression. For example, paternally derived genes will be less related to an individual's mother than will maternally derived genes, and so will usually be selected to extract more maternal investment (Haig & Westoby 1989; Moore & Haig 1991). Indeed, Haig (1992) has shown that the optimal level of maternal investment will generally differ between maternal genes, maternally derived offspring genes, paternally derived offspring genes, and unimprinted offspring genes (see also Queller 1994; Haig 1996). In this paper we show that these differing optima will also apply to the evolution of the imprinting machinery itself.

Three different classes of gene are likely to be involved in any particular instance of genomic imprinting: the imprinted genes themselves, *trans*-acting genes in the parents, which affect the application of the imprint, and *trans*-acting genes in the offspring, which recognize and affect the expression of the imprint (Efstratiadis 1994). We show that coefficients of relatedness between individuals will typically differ among these three classes, thus engendering conflicts of interest between Imprinter genes, imprinted genes, and imprint-recognition genes.

As a disproportionate number of imprinted genes in mice and humans are involved in placental and juvenile growth (Barlow 1995), we will again consider the example of interactions between mother and offspring. In addition, we use Hamilton's Rule (Hamilton 1963, 1964*a*,*b*), restricting ourselves to the simplest case of panmictic populations with weak selection, for which coefficients of relatedness can be simply derived from genealogical relationships (Grafen 1985). Conflicts between different classes of genes are demonstrated by showing that different conditions for the spread of a new mutation apply to the different classes (Trivers 1974).

2. CONFLICTS IN THE MATERNAL GERM LINE

Maternally derived genes in a juvenile (or placenta) are, by definition, found in the mother with probability 1. Therefore, for a locus (e.g. a growth promoter) that is initially silent, a new mutant that is active if inherited from the mother will be selected for only if the benefit it brings to the offspring expressing it (b_o) is greater than the cost it incurs to the mother (c_m): $b_o > c_m$, (figure 1*a*) (benefits and costs refer to changes in the reproductive value (RV) of individuals (Hamilton 1966; Charlesworth & Charnov 1981); in populations of constant size, this is equivalent to changes in the expected number of offspring). The same condition applies for a locus (e.g. a growth suppressor) that is biallelically expressed, and a new mutant that is silent if inherited from the mother. However, now consider an Imprinter gene that acts in *trans* in the maternal germ line to apply the imprint. The probability of it being inherited along with the imprint is 1/2 (that is, the offspring expressing the imprint is related to the Imprinter allele by a coefficient of 1/2), and so a new mutant will spread only if the benefit to the offspring

*Author for correspondence (a.burt@ic.ac.uk).

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is more than twice the cost to the mother ($b_o > 2c_m$). Therefore, if the benefit of a maternally imprinted gene to the offspring expressing it is in the range $c_m < b_o < 2c_m$, then there will be a conflict of interest, with the target gene being selected to acquire the imprint and the Imprinter gene being selected not to apply it.

Conflicts between Imprinter genes and target genes can also arise in the opposite direction, for altruistic mutations that benefit the mother at the expense of the offspring expressing them (figure 1*b*). That is, for a locus (e.g. a growth suppresser) that is initially silent, a new mutant that is active if inherited from the mother will increase in frequency only if the benefit to the mother (b_m) is more than the cost to the offspring (c_o): $b_m > c_o$ (and similarly for a growth promoter locus that is biallelically expressed and a new mutant that is silent if maternally inherited). However, a *trans*-acting Imprinter gene will be selected to apply the imprint as long as $b_m > c_o/2$. If $c_o/2 < b_m < c_o$, then again there will be a conflict of interest, except that now the Imprinter gene will be selected to apply the imprint, and the target gene selected to avoid it.

3. CONFLICTS IN THE PATERNAL GERM LINE

Such conflicts between Imprinter loci and their targets may also arise in the paternal germ line. Here, selection on genes affecting maternal investment will depend critically upon how costs borne by the mother affect the father. Suppose a unit change in maternal RV has effect k on the father's value. Then, for a growth promoter that is initially silent, a new mutation that is active if inherited from the father will increase in frequency if $b_o > kc_m$. However, a *trans*-acting paternal Imprinter gene will only be selected to apply the imprint if $b_o > 2kc_m$. Thus, if $kc_m < b_o < 2kc_m$, then there will be a conflict, with the growth gene being selected to acquire the imprint and the Imprinter gene being selected not to apply it. Again, conflicts with the opposite orientation arise for altruistic mutations, when $c_o/2 < kb_m < c_o$.

Note that conflicts only arise in the paternal germ line when $k > 0$ (figure 1). What is this parameter? As defined, it measures how fitness effects on mothers affect fathers. Thus, its value will clearly depend on the mating system: $k = 0$ with complete promiscuity and $k = 1$ with lifetime monogamy. With polygyny, $k = 1$ if there is no interference between females; it will be less than 1 if there is interference (so that if one female suffers a cost, another female will replace at least part of the loss), and greater than 1 if there are synergistic or cooperative effects between females in a harem. Note that in the latter case, one would expect the usual imprinting asymmetries to reverse, with growth suppressers being paternally expressed. Note too that our general approach of attributing the costs of offspring selfishness directly to parental RV is apparently novel, contrasting with the more usual approach in which these costs are borne by maternal siblings, either extant or future, which may or may not have the same father ($r = 1/2$ or $1/4$, respectively; see, for example, Trivers 1974). However, this approach can be misleading, or at least difficult to apply correctly (Mock & Parker 1997, pp. 151–154). Thus, Haig (1992, 1996) suggests that the conflict between maternally and

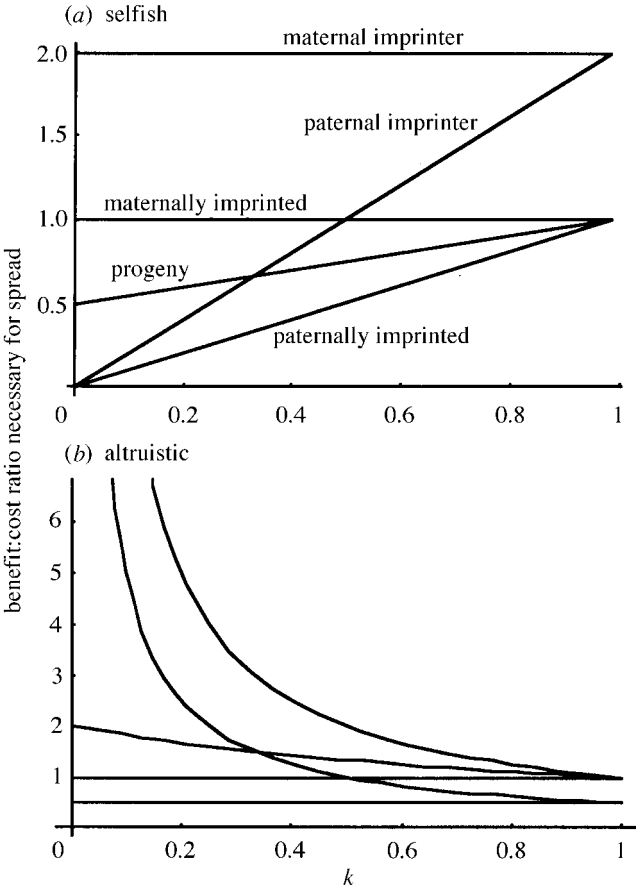

Figure 1. Threshold benefit–cost ratios necessary for the spread of a gene as a function of k, the effect on paternal reproductive value of a unit change in the maternal value, for different types of genes. (*a*) Genes that increase offspring growth (benefits to offspring, costs to mother); (*b*) genes that reduce offspring growth (benefits to mother, costs to offspring; labelling of lines is in inverse order to that above). Values in (*a*) also give the stopping rule for maternal investment for the different types of genes. Note that for $k = 1$, all conflict is parent–offspring, and that as k gets smaller, the conflict gradually becomes more maternal–paternal.

paternally derived genes disappears if females only mate with one male in their lifetime, but this is unlikely to be generally true. Even if females are monandrous, if offspring selfishness causes reduced maternal survival, and if a male is able to replace a dead mate, then $k < 1$ and paternally derived genes will be selected to extract more maternal investment than maternally derived genes. In an analysis of parent–offspring relations, our approach seems the more direct and less likely to lead to errors. (Even so, the relation between maternal and paternal RV may be more complicated than assumed here: for example, decrements in maternal survival will have less effect on paternal RV than decrements in maternal fertility if a dead mate is replaced more easily than a subfertile mate. However, these complications need not concern us here.)

4. CONFLICTS IN THE OFFSPRING

Supposing that the maternally and paternally derived genes in a newly formed zygote are differentially

imprinted, this does not guarantee differential expression, for the imprint must be inherited through the many mitoses in offspring development and must have some effect on gene expression. Both requirements will involve the action of *trans*-acting genes in the offspring, to maintain and read the imprint. Assuming that these genes are not themselves imprinted, they will have yet another threshold for the spread of a new mutation, namely that $b_o > (1+k)c_m/2$ for a growth promoter, and $b_m > 2c_o/(1+k)$ for a growth suppresser, assuming they are autosomal (figure 1). Thus, even if a gene is selected to acquire an imprint, and Imprinter genes selected to apply it, offspring genes may nonetheless be selected to remove the imprint or to ignore it. Imprints might easily be removed by changing patterns of methylation (Chaillet *et al.* 1995), and they can be ignored by, for example, starting transcription in a different place, as occurs for human *IGF2* when it switches from paternal expression in foetal liver to biallelic expression in adult liver (Vu & Hoffman 1994). This switch is presumably under the control of *trans*-acting transcription factors. Thus, intragenomic conflicts may arise both over the application of an imprint in the parental germ lines and over the expression of an imprint in the offspring.

5. DISCUSSION

Patterns of relatedness will often differ for maternally and paternally derived genes, and this asymmetry is a likely source of natural selection for parent-specific gene expression (Haig & Westoby 1989; Moore & Haig 1991). This maternal–paternal conflict was first described as depending upon females having more than one mate; as we have noted above, it more accurately depends upon decrements in the female's RV being, from the male's point of view, replaceable. Previous models of this kinship theory of imprinting have considered the evolution of *cis*-acting control regions affecting the level of imprinting (Haig 1992, 1996; Mochizuki *et al.* 1996; Spencer *et al.* 1998). We have extended these analyses to the other sorts of genes likely to be involved in genomic imprinting, *trans*-acting genes in the parental germ lines and *trans*-acting genes in the offspring, and have demonstrated that there will be well-defined conflicts of interest between these different classes of gene. Spencer & Williams (1997) have previously presented models for the evolution of imprinting with *cis*- and *trans*-acting germ line modifiers, but did not consider traits in which kinship is important, and so did not find a conflict. The existence of conflicts between Imprinter and imprinted loci, and between imprinted and imprint-recognition loci, could have a number of important consequences for the evolution of genomic imprinting.

First, conflicts of interest of the sort described here could lead to a constantly dynamic pattern of perpetual selection, as evolutionary change in one component of the imprinting machinery selects for an evolutionary response by another. As noted above, conflicts in the parental germ lines can be in either direction, each with its expected evolutionary dynamic. First, a locus may be selected to acquire an imprint while an Imprinter is selected to not apply it. In this case, the target gene may, for example, be selected to mimic other imprinted genes, by acquiring their recognition sequences, and the imprinting apparatus therefore selected to make ever finer discriminations between genes it wants to imprint and those it does not. Alternatively, an Imprinter may be selected to apply an imprint and the target selected to avoid it, in which case the imprinting apparatus will be chasing the target locus through sequence space. Antagonistic coevolution may also occur between imprinted genes and imprint-recognition genes. One possible manifestation of such perpetual selection would be a breakdown of the normal pattern of parent-specific gene expression in species hybrids and back-crosses. Vrana *et al.* (1998) have shown that expression of some imprinted genes differs between reciprocal F1 hybrids of *Peromyscus maniculatus* and *P. polionotus*, indicating there has been recent evolution of these genes and/or of the imprint recognition machinery since the species diverged. Interestingly, not all imprinted genes showed the same pattern in the reciprocal hybrids; this result indicates that parent-specific expression of different loci is under at least somewhat separate control.

Second, not all genes whose evolution might be affected by asymmetric coefficients of relatedness have parent-specific expression (e.g. *Igf1*, a gene affecting foetal growth). Why not? Haig (1997) suggests that this may simply be due to the absence of appropriate mutations, Mochizuki *et al.* (1996) suggest that it may be due to the increased expression of deleterious recessives at imprinted loci, and Spencer *et al.* (1998) suggest that it may be because the costs to the mother outweigh the benefits to the offspring. As an alternative, we suggest that the different components of the imprinting machinery will not always be selected in the same direction (especially for maternal imprinting; see below), and parent-specific expression may not evolve because the 'nays' have won.

Third, conflicts within the imprinting machinery may also help explain why imprinted genes tend to occur in clusters (reviewed by Reik & Maher (1997)) as follows: if there is a conflict between a gene selected to acquire an imprint and Imprinter genes selected not to apply it, perhaps the former can evolve to make use of mechanisms operating at other nearby loci where Imprinters and targets are both positively selected. Consistent with this idea, there appear to be complex interconnected causal pathways acting both in *cis* and in *trans* within these clusters (Buiting *et al.* 1995; Dittrich *et al.* 1996; Forné *et al.* 1997; Webber *et al.* 1998). Previous explanations for clustering have posited a lack of genetic variation for becoming imprinted (Haig 1997) or an imprinting process that is costly (Mochizuki *et al.* 1996).

Fourth, Moore & Reik (1996) have suggested that such conflicts could account for the complex pattern of de- and remethylation observed at some imprinted loci. For example, at a particular cytosine of *Igf2r*, the maternal copy is methylated at the zygote and two-cell stage, is unmethylated at the four-cell stage, then reacquires methylation at the eight-cell stage (Razin & Shemer 1995; Shemer *et al.* 1996). Perhaps even the genome-wide demethylation that occurs early in mouse development (Li 1997) is the organism's attempt to reduce the frequency of unwanted imprints, both maternal and paternal.

Fifth, recognition of conflicts in the imprinting machinery gives reasons for thinking that paternal

imprinting may be more common than maternal imprinting, as follows. Each of the five classes of gene we have discussed will have a different optimal level of maternal investment: in particular, each class of gene will be selected to continue investment until the marginal benefit-to-cost ratio falls below the corresponding value in figure 1*a*. The actual amount of investment at any point in evolutionary time seems likely to be intermediate between the various optima, perhaps closest to the maternal Imprinter optimum because maternal genes have so much more control than other genes over maternal investment. If so, then it will be between the optima for maternal Imprinter genes and maternally imprinted genes, and so these will be selected to change investment in opposite directions, maximizing the conflict of interest. On the other hand, both paternal Imprinter genes and paternally imprinted genes will often be selected to increase the level of maternal investment, particularly when k is small, and so are less likely to disagree. Imprints arising in the paternal germ line are also more likely to be in the offspring's interest, and so are more likely to be maintained and used. Such reasoning suggests that imprinting may be more stable over evolutionary time in the paternal germ line than in the maternal germ line, and that paternally imprinted genes should therefore come to outnumber maternally imprinted genes.

Other lines of reasoning lead to the same prediction: (i) mothers have many ways to influence maternal investment other than via imprinting, whereas fathers are much more limited in their options; and (ii) the actual level of investment is likely to be further away from the paternal optima than from the maternal optima, and so selection for change will be stronger. Against these considerations must be weighed the greater influence maternal genes can have over imprinted loci, through cytoplasmic RNAs in the oocyte (Latham & Sapienza 1998), which could lead to a preponderance of maternally imprinted genes.

Unfortunately, we do not know for most imprinted genes whether the imprinting is maternal or paternal. Expression patterns cannot easily be used to decide because imprinting may be the parent-specific silencing of an allele that used to be active, or the activation of an allele that used to be silent. For example, *Ins1* and *Ins2* in mice are paternally expressed in the yolk sac, but biallelically expressed in the pancreas, and it is not yet clear whether the evolutionary innovation was silencing of the maternal allele (maternal imprinting; the ancestral state was biallelic expression in the yolk sac) or activation of the paternal allele (paternal imprinting; the ancestral state was no expression in the yolk sac). Similarly, methylation patterns cannot be used to decide because imprinting may be the addition of methyl groups that are usually absent, or the removal of ones that are usually present (Chaillet *et al.* 1995). Rather, comparative and genetic studies are required to determine what type of gene changed, and in which germ line the change occurred, in the evolution of parent-specific expression.

The five classes of gene discussed thus far do not exhaust the possibilities. For example, Imprinter genes might themselves be imprinted and work in a parent-specific manner, in which case one would have to consider relatedness over three generations. This is perhaps not too far-fetched, as genes that affect the methylation of artificially constructed transgenes have been shown to work in a parent-specific manner (Allen & Mooslehner 1992). Sex-linked genes can also have optima for kin-selected traits that are different from those of autosomal genes, and hence their own set of benefit–cost thresholds (Hamilton 1972). This will almost certainly be the case if the effects on relatives are sex-specific (e.g. foetal testosterone production, which is good for brothers and bad for sisters (Clark & Galef 1995)). Considering only the case where litter-mates have the same father, autosomes and maternally derived Xs in a female foetus are related to all litter-mates by a factor of 1/2, but the paternally derived X is related to sisters by a factor of 1 and to brothers by a factor of 0. Thus, paternally derived Xs will be selected to produce a relatively female-beneficial, male-detrimental uterine environment, as will X-linked Imprinter genes active in the male germ line, whereas maternally derived Xs and all autosomes will be selected to produce a more gender-neutral foetal environment. The result will be conflicts between different components of the imprinting apparatus, and between different components of the imprint-recognition apparatus, if some of the genes involved are sex-linked and others autosomal.

Conflicts of interest over imprinting do not depend upon maternal–paternal asymmetries, for even if $k=1$, parental Imprinter loci may be selected to apply an imprint (e.g. inactivate a growth enhancer, or activate a growth suppresser), while target loci and imprint-recognition loci are selected to lose it (figure 1). Similarly, they need not be limited to foetal characters; indeed, it is difficult to think of any class of interaction between relatives that will not produce such conflicts, including alarm calls, dispersal, dormancy, inbreeding and inbreeding avoidance, helping to raise siblings, etc. (Trivers & Burt 1998). On the other hand, imprinting itself need not be limited to kin-selected traits: if there is selection for a change in gene dosage or tissue-specificity and the first appropriate mutation happens to work in a parent-specific manner, then it may be selected for and go to fixation. One possible example is the demethylation of *Xist* in the paternal germ line of mice, which apparently marks the X chromosome for inactivation in the offspring trophectoderm (Norris *et al.* 1994; Ariel *et al.* 1995; Zuccotti & Monk 1995). By inactivating the X chromosome, the male is making it match the degenerate Y chromosome transmitted in the other half of his gametes, a simple form of dosage compensation available only to taxa with methylation (so, for example, not *Drosophila* or *Caenorhabditis*). Imprinting of genes unrelated to kin selection will not lead to intragenomic conflicts.

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REFERENCES

Allen, N. D. & Mooslehner, K. A. 1992 Imprinting, transgene methylation and genotype-specific modification. *Semin. Dev. Biol.* **3**, 87–98.

Ariel, M., Robinson, E., McCarrey, J. R. & Cedar, H. 1995 Gamete-specific methylation correlates with imprinting of the murine *Xist* gene. *Nature Genet.* **9**, 312–315.

Barlow, D. P. 1995 Gametic imprinting in mammals. *Science* **270**, 1610–1613.

Buiting, K., Saitoh, S., Gross, S., Dittrich, B., Schwartz, S., Nicholls, R. D. & Horsthemke, B. 1995 Inherited microdeletions in the Angelman and Prader-Willi syndromes define an imprinting centre on human chromosome 15. *Nature Genet.* **9**, 395–400.

Chaillet, J. R., Bader, D. S. & Leder, P. 1995 Regulation of genomic imprinting by gametic and embryonic processes. *Genes Devel.* **9**, 1177–1187.

Charlesworth, B. & Charnov, E. L. 1981 Kin selection in age-structured populations. *J. Theor. Biol.* **88**, 103–119.

Clark, M. M. & Galef, B. G. J. 1995 Prenatal influences on reproductive life history strategies. *Trends Ecol. Evol.* **10**, 151–153.

Dittrich, B. (and 10 others) 1996 Imprint switching on human chromosome 15 may involve alternative transcripts of the *SNRPN* gene. *Nature Genet.* **14**, 163–170.

Efstratiadis, A. 1994 Parental imprinting of autosomal mammalian genes. *Curr. Opin. Genet. Devel.* **4**, 265–280.

Forné, T., Oswald, J., Dean, W., Saam, J. R., Bailleul, B., Dandolo, L., Tilghman, S. M., Walter, J. & Reik, W. 1997 Loss of the maternal *H19* gene induces changes in *Igf2* methylation in both cis and trans. *Proc. Natn. Acad. Sci. USA* **94**, 10 243–10 248.

Grafen, A. 1985 A geometric view of relatedness. *Oxf. Surv. Evol. Biol.* **2**, 28–89.

Haig, D. 1992 Genomic imprinting and the theory of parent–offspring conflict. *Semin. Dev. Biol.* **3**, 153–160.

Haig, D. 1996 Placental hormones, genomic imprinting, and maternal–fetal communication. *J. Evol. Biol.* **9**, 357–380.

Haig, D. 1997 Parental antagonism, relatedness asymmetries, and genomic imprinting. *Proc. R. Soc. Lond.* B **264**, 1657–1662.

Haig, D. & Westoby, M. 1989 Parent-specific gene expression and the triploid endosperm. *Am. Nat.* **134**, 147–155.

Hamilton, W. D. 1963 The evolution of altruistic behaviour. *Am. Nat.* **97**, 354–356.

Hamilton, W. D. 1964*a* The genetical evolution of social behaviour. I. *J. Theor. Biol.* **7**, 1–16.

Hamilton, W. D. 1964*b* The genetical evolution of social behaviour. II. *J. Theor. Biol.* **7**, 17–52.

Hamilton, W. D. 1966 The moulding of senescence by natural selection. *J. Theor. Biol.* **12**, 12–45.

Hamilton, W. D. 1972 Altruism and related phenomena, mainly in social insects. *A. Rev. Ecol. Syst.* **3**, 193–232.

Latham, K. E. & Sapienza, C. 1998 Localization of genes encoding egg modifiers of paternal genome function to mouse chromosomes one and two. *Development* **125**, 929–935.

Li, E. 1997 Role of DNA methylation in mammalian development. In *Genomic imprinting* (ed. W. Reik & A. Surani), pp. 1–20. Oxford University Press.

Mochizuki, A., Takeda, Y. & Iwasa, Y. 1996 The evolution of genomic imprinting. *Genetics* **144**, 1283–1295.

Mock, D. W. & Parker, G. A. 1997 *The evolution of sibling rivalry.* Oxford University Press.

Moore, T. & Haig, D. 1991 Genomic imprinting in mammalian development: a parental tug-of-war. *Trends Genet.* **7**, 45–49.

Moore, T. & Reik, W. 1996 Genetic conflict in early development: parental imprinting in normal and abnormal growth. *Rev. Reprod.* **1**, 73–77.

Norris, D. P., Patel, D., Kay, G. F., Penny, G. D., Brockdorff, N., Sheardown, S. A. & Rastan, S. 1994 Evidence that random and imprinted *Xist* expression is controlled by preemptive methylation. *Cell* **77**, 41–51.

Queller, D. C. 1994 Male–female conflict and parent–offspring conflict. *Am. Nat.* **144**, S84–S99.

Razin, A. & Shemer, R. 1995 DNA methylation in early development. *Hum. Molec. Genet.* **4**, 1751–1755.

Reik, W. & Maher, E. A. 1997 Imprinting in clusters: lessons from Beckwith–Wiedemann syndrome. *Trends Genet.* **13**, 330–334.

Reik, W. & Surani, A. (eds) 1997 *Genomic imprinting.* Oxford University Press.

Shemer, R., Birger, Y., Dean, W. L., Reik, W., Riggs, A. D. & Razin, A. 1996 Dynamic methylation adjustment and counting as part of imprinting mechanisms. *Proc. Natn. Acad. Sci. USA* **93**, 6371–6376.

Spencer, H. G. & Williams, M. J. M. 1997 The evolution of genomic imprinting: two modifier-locus models. *Theor. Popul. Biol.* **51**, 23–35.

Spencer, H. G., Feldman, M. W. & Clark, A. G. 1998 Genetic conflicts, multiple paternity and the evolution of genomic imprinting. *Genetics* **148**, 893–904.

Trivers, R. L. 1974 Parent–offspring conflict. *Am. Zool.* **14**, 249–264.

Trivers, R. & Burt, A. 1998 Kinship and genomic imprinting. In *Genomic imprinting* (ed. R. Ohlsson), pp. 1–21. Heidelberg: Springer.

Vrana, P. B., Matteson, P., Guan, X.-J., Ingram, R. S. & Tilghman, S. M. 1998 Genomic imprinting is disrupted in interspecific *Peromyscus* hybrids. *Nature Genet.* (In the press.)

Vu, T. H. & Hoffman, A. R. 1994 Promoter-specific imprinting of the human insulin-like growth factor-II gene. *Nature* **371**, 714–717.

Webber, A. L., Ingram, R. S., Levorse, J. M. & Tilghman, S. M. 1998 Location of enhancers is essential for the imprinting of *H19* and *Igf2* genes. *Nature* **391**, 711–715.

Zuccotti, M. & Monk, M. 1995 Methylation of the mouse *Xist* gene in sperm and eggs correlates with imprinted *Xist* expression and paternal X-inactivation. *Nature Genet.* **9**, 316–320.