Sexual Antagonism and the Evolution of X Chromosome Inactivation

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X-CHROMOSOME INACTIVATION

SELF-IMPOSED SILENCE: PARENTAL ANTAGONISM AND THE EVOLUTION OF X-CHROMOSOME INACTIVATION

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Abstract.— A model is proposed for the evolution of X chromosome inactivation (XCI) in which natural selection initially favors the silencing of paternally-derived alleles of X-linked demand inhibitors. The compensatory upregulation of maternally-derived alleles establishes a requirement for monoallelic expression in females. For this reason, XCI is self-reinforcing once established. However, inactivation of a particular X chromosome is not. Random XCI (rXCI) is favored over paternal XCI because rXCI reduces the costs of functional hemizygosity in females. Once present, rXCI favors the evolution of locus-by-locus imprinting of X-linked loci which creates an evolutionary dynamic in which different chromosomes compete to remain active.

Key words: dosage compensation, genomic imprinting, parental antagonism, sexual antagonism, X-chromosome inactivation

Female mammals possess two X chromosomes, one inherited from each parent. Males, on the other hand, possess a single X inherited from their mother. Despite this two-fold difference in gene dosage, a single X-linked allele is expressed in XX as well as XY nuclei, because one of the X chromosomes in XX nuclei of differentiated cells is transcriptionally inactive (Plath et al. 2002).

The function of X chromosome inactivation (XCI) is commonly assumed to be dosage compensation. That is, XCI is believed to have evolved as a mechanism to equalize the level of expression of X-linked loci in the two sexes. XCI is a strange way to equalize expression, however, because females forgo some of the advantages of having two functional copies of each X-linked locus. In *Caenorhabditis* and *Drosophila*, dosage is compensated without this disadvantage by the simple expedient of having the single X of males expressed at twice the level of each X chromosome of females (Parkhurst and Meneely 1994). XCI is also a strange mechanism in that homologous, sometimes identical, sequences have different expression in the same nucleus. XCI shares both of these peculiar features with genomic imprinting and an evolutionary connection between the two epigenetic phenomena has often been suggested (e.g., Ohlsson et al. 2001; Lee 2003a; Huynh and Lee 2005; Reik and Lewis 2005)

Five considerations suggest that genomic imprinting and XCI may be conceptually and mechanistically related. First, both are processes that forgo the partial protection that diploidy provides against the effects of deleterious somatic or germline mutations (Sapienza 1989; Charlesworth 1996). Second, both are processes by which different copies of a DNA sequence have different behaviors within a single nucleus (Hendrich and Willard 1995). Third, parental origin determines which X chromosome is inactivated

in marsupials (Cooper et al. 1971; Sharman 1971) and extraembryonic tissues of cattle (Xue et al. 2002), rats (Wake et al. 1976), and mice (Takagi and Sasaki 1975). Fourth, the kinds of genes that are predicted to be subject to imprinting are also the kinds of genes for which dosage compensation should be important. That is, both processes are expected to have evolved because of genes whose effects are dosage sensitive. For most loci at which the effects of loss-of-function mutations show recessive inheritance, effects on the phenotype are dosage-insensitive and there would be only weak selection for dosage compensation, just as there would be only weak selection for imprinting (Haig 1997). Fifth, the mechanisms of XCI and imprinted allele inactivation share common molecular machinery (Lee 2003a; Mager et al. 2003; Silva et al. 2003).

Haig and others (see papers collected in Haig 2002) have argued that genomic imprinting has evolved in mammals because of conflicting selective forces acting on genes of maternal and paternal origin. Situations in which selection acts differentially with respect to parental origin have been termed *parental antagonism* by analogy to sexual antagonism which arises when a gene's expression benefits one sex but not the other (Haig 1997). Parental antagonism arises in the context of interactions with kin that are unequally related to an individual via the individual's mother and father. Interactions subject to parentally-antagonistic selective forces include, but are not restricted to, an individual's interaction with its parents. The evolution of parental origin-limited gene expression at a locus subject to parental antagonism is analogous to the evolution of sex-limited expression at a locus subject to sexual antagonism (Haig 1997).

One situation in which matrilineal and patrilineal interests diverge is over the level of demand that offspring impose on their mothers, with patrilineal interests favoring higher

levels of demand than matrilineal interests. The balance of forces differs between X-linked and autosomal loci because all eggs, but only 50% of sperm, carry an X chromosome, whereas every gamete carries a copy of each autosome. As a consequence, X-linked genes are predicted to display an evolutionary bias favoring matrilineal interests, including lower levels of demand by offspring for maternal resources (Haig 2000a, 2000b, 2006). For this reason, Haig (2000a) suggested that imprinted (paternal) X inactivation may have evolved because paternally-derived X-linked alleles gained an advantage from shutting down their own chromosome to reduce the effective dosage of demand inhibitors, thus favoring patrilineal interests over matrilineal interests. The current paper develops this hypothesis in greater detail.

EVOLUTION OF X INACTIVATION

Preliminaries

The model that will be presented in subsequent sections is based on the premise that, prior to the origin of XCI, genes on the X chromosome functioned to reduce the level of demand that offspring imposed on their mothers. Haig (2006) has called this the hypothesis of *X-Linked Inhibitory Bias* (XLIB). In that paper, I present reasons for this theoretical prediction and review evidence that bears on this prediction. Briefly, genes on the X chromosome are predicted to favor maternal interests because alleles at X-linked loci are maternally-derived two-thirds of the time by contrast to alleles at autosomal loci that are maternally-derived half of the time (also see Mills and Moore 2006). Relevant data are limited and XLIB is neither strongly supported nor refuted. The present paper does not repeat these arguments but develops a scenario for the evolution of XCI given the assumption that XLIB is correct. The scenario addresses the evolution of expression

levels in females but does not consider the evolution of expression levels in males. First, I use the predicted bias in favor of demand inhibitors on X chromosomes to develop a model for the origin of paternal X chromosome inactivation (pXCI) and then present a model for the subsequent origin of random X chromosome inactivation (rXCI). If XLIB is refuted, the scenario will fail.

In the absence of genomic imprinting, the aggregate level of expression (from both alleles) of a demand inhibitor is predicted to be a compromise between a higher level favored when an allele is maternally derived and a lower level favored when the allele is paternally derived. If the locus is subject to genomic imprinting, the evolutionarily stable strategy (ESS) for an allele is to be expressed at the higher (maternal) optimum when maternally derived but to be silent when paternally derived. This prediction has been termed the "loudest-voice-prevails" (LVP) principle. The prediction that one allele should be silenced at the ESS presupposes that maternally-derived and paternally-derived alleles of a diploid individual contribute their gene product to a common pool, the size of which determines effects on fitness (Haig 1997, 2006). The principle does not apply to genes with cell-autonomous effects that are subject to rXCI because then maternally-derived and paternally-derived alleles are expressed in different cells.

I will use the adjectives *madumnal* to refer to maternally-derived alleles or chromosomes and *padumnal* to refer to paternally-derived alleles or chromosomes. Haig (1996) introduced this terminology because ambiguities can arise in discussions of the evolution of genomic imprinting if maternal and paternal can be used to refer *either* to genes present in mothers and fathers *or* to genes derived from mothers and fathers present in offspring. Queller (2003) has suggested the more euphonious *matrigenic* and

patrigenic to make the same distinction. I am happy for popular usage to determine which pair of terms, if either, is broadly adopted.

Origin of padumnal X inactivation

In the presence of genomic imprinting, natural selection favors the inactivation of padumnal alleles at demand-inhibitor loci and an associated up-regulation of madumnal alleles to more than compensate for padumnal silence (Haig 2006). This immediately suggests an evolutionary model for the origin of padumnal X-chromosome inactivation (pXCI). Natural selection favored reduced expression of the padumnal alleles of X-linked demand inhibitors because this action increased demand while at the same time reducing the component of cost due to production of the demand inhibitors. Two variants of the model can be considered: *coalescence* of inactivation from multiple centers or *diffusion* of inactivation from a single center. Under the first variant, padumnal inactivation evolved independently at multiple demand inhibitor loci. Inactivation then spread to intervening loci, perhaps for reasons of mechanistic efficiency and chromatin structure, or for reasons of dosage compensation. Under the second variant, natural selection favored a padumnally-expressed agent within the X-linked block that caused the down-regulation of loci in *cis*.

The proposed advantage to an agent on the padumnal X (X^P) of inactivating its own chromosome would also apply if the agent inactivated the madumnal X (X^M), or a random X, instead. Inactivation of X^M or a random X would have been evolutionarily less stable than inactivation of X^P, however. This is because genes on X^P would have benefited from reduced expression of X-linked demand inhibitors, whereas genes on X^M

would have been disadvantaged by reduced expression of demand inhibitors. X-linked genes would therefore be selected to avoid inactivation when maternally-derived but to facilitate inactivation when paternally-derived.

Unimprinted autosomal genes (or padumnally-expressed autosomal genes) would also have benefited from decreased expression of X-linked demand inhibitors, because autosomal loci are not subject to the matrilineal bias of X-linked loci. However, I favor a hypothesis in which the primary mutations occurred on the X chromosome because an autosomal location would not explain specificity of inactivation for X^P. (In the absence of genomic imprinting of X-linked loci there would be no reason why *trans*-acting factors should discriminate between X chromosomes on the basis of parental origin.) Nevertheless, natural selection on autosomal loci is unlikely to have opposed the early stages in the evolution of pXCI.

The short-term effect of the origin of pXCI would be to reduce the expression of X-linked demand inhibitors. The long-term effect would be the opposite. Before pXCI, the expression of a demand inhibitor is a matrilineally-weighted compromise between madumnal and padumnal interests. Under the diffusion model, one locus, the imprinted *cis*-inactivator on the padumnal X, is subject to selection solely on its effects when paternally-derived whereas the inactivated loci are subject to selection solely on their effects when maternally derived. The change in selective forces that accompanies the origin of pXCI favors increased expression of X-linked demand inhibitors, and intensifies selection on the *cis*-inactivator to prevent reactivation of its own chromosome. That is, the LVP principle predicts that the upregulation of madumnal alleles of X-linked demand inhibitors proceeded in concert with the downregulation of padumnal alleles. The

resultant high-level of madumnal expression would have created a selective force against the reactivation of padumnal alleles. Moreover, the evolutionary adjustment of expression levels in the context of a single active X would provide a selective force acting across all loci, both autosomal and X-linked, against reactivation of the silent X. As additional loci became subject to XCI—whether or not the loci were involved in the inhibition of demand—natural selection would adjust the expression level of their single active allele in females to accommodate functional hemizygosity at the locus, further reinforcing selection against reactivation of the inactive X.

The above scenario assumes that pXCI evolved from a state in which both X chromosomes were active in females, but is not contingent on the presence or absence in this ancestral mammal of a pre-existing form of dosage compensation, nor on the presence or absence of functional homologs of X-linked alleles on the Y chromosome. All that is required is that there had been sufficient selection on X-linked loci for these to show a matrilineal bias in their effects and that some of these parentally-antagonistic effects were dosage sensitive. Given these conditions, natural selection would favor padumnally-expressed factor(s) that reduced expression of X-linked loci.

The model would be unable to explain the evolution of pXCI from rXCI. If dosage compensation by rXCI were already in existence, and X-linked loci were unimprinted, a padumnally-expressed agent would gain nothing in terms of reduced expression of demand inhibitors by replacing rXCI by pXCI. If there were significant pre-existing imprinting of X-linked loci, X^P would be a weaker demand inhibitor than X^M. Thus, there would be no incentive for an imprinted agent on either X to shut its own chromosome

down, although there would be a potential advantage for agents on X^P to shut down X^M, and vice versa.

Phylogeny of X inactivation

Phylogenetic comparisons currently provide little evidence with which to test the prediction that pXCI is ancestral to rXCI. pXCI has been described from both Australian and New World marsupials (Cooper et al. 1993) and therefore is probably ancestral for all living marsupials. rXCI is often tacitly assumed to occur in all eutherian mammals but, has in fact, been described in only a few species. The best evidence comes from mice and humans. Recent phylogenies place both species in the Euarchontoglires, one of four major clades of extant eutherian mammals (Murphy et al. 2001). Good evidence also exists for rXCI in cats (Lyon 1974) and cattle (Xue et al. 2002), both members of the Laurasiatheria. Thus, rXCI appears to have been present in the common ancestor of Boreoeutheria, the clade that unites Euarchontoglires and Laurasiatheria (Amrine-Madsen et al. 2003). Data for the remaining major clades of eutherian mammals are very limited. Jegalian and Page (1998) observed methylation of the CpG island associated with X-linked *ALD* in female, but not in male, elephants (Afrotheria) and giant anteaters (Xenarthra). They interpreted this sex difference as evidence for XCI in these species but had no information about which X was inactive. Available evidence on XCI in monotremes—even on the basic question of whether or not XCI occurs—is sufficiently sketchy to allow few inferences about the ancestral state of XCI in the monotreme-therian ancestor, or to use monotremes as an outgroup to make inferences about the nature of XCI in the marsupial-eutherian ancestor (for reviews see Cooper et al. 1993; Graves

1996). On the basis of the known phylogenetic distribution of the two kinds of inactivation, it would be equally parsimonious to conclude that pXCI was ancestral to rXCI, or the reverse.

Data on molecular mechanisms are little help in resolving the evolutionary relationship between marsupial pXCI and eutherian rXCI, because nothing substantive is known about the mechanism of pXCI in marsupials. Some X-linked loci of marsupials show partial escape from pXCI in a complex pattern that differs among loci, tissues, and species (Cooper et al. 1993). This variability is perhaps more consistent with a model in which pXCI in marsupials has evolved independently at multiple loci than one in which there is a single center of XCI.

The selective forces that have been proposed to favor the evolution of genomic imprinting and pXCI—namely a protracted relation between mother and offspring—appear to exist in both monotremes and marsupials. The principal demands of offspring upon mothers occur during lactation in these groups, and thus it is principally inhibitors of lactational demand that are predicted to be concentrated on the X chromosome. "Placental" demands however do potentially exist in both groups because the extraembryonic membranes of monotreme eggs absorb nutrients *in utero* (Hughes 1993) and marsupial fetuses possess a yolk sac placenta (Harder et al. 1993). *Igf2* and *Igf2r* are the first genes to have been tested for imprinting in monotremes and marsupials. Neither gene appears to be imprinted in monotremes, whereas both genes are imprinted in marsupials (Killian et al. 2000, 2001a, 2001b; O'Neill et al. 2000). Further research will be necessary to uncover the genetic basis of lactational demand and to determine the chromosomal location and expression patterns of nursing-related genes in these groups.

Origin of random X inactivation

Although selection for inactivation of an X chromosome, once established, is self-reinforcing, selection for inactivation of a particular X is not. Padumnally-silent loci are never subject to selection for their effects when paternally derived. Therefore, an X^P released from pXCI would *initially* be functionally interchangeable with an active X^M and its expression would serve matrilineal interests (Moore et al. 1995). All genomic factions would at first benefit from replacing pXCI with random X-chromosome inactivation (rXCI) because the independent, random inactivation of X^M and X^P in different cell lineages would restore some of the advantages of functional heterozygosity for X-linked genes (Charlesworth 1996). The replacement of pXCI by rXCI however would mean that X-linked loci were now subject to selection on their effects when paternally derived. This would create an opportunity for the evolution of locus-by-locus imprinting on the X chromosome. Natural selection would favor the down-regulation of the padumnal alleles of X-linked demand inhibitors, but not necessarily their silencing, because the LVP principle does not apply at loci subject to rXCI. Therefore, imprinting of loci subject to rXCI may show quantitative differences between madumnal and padumnal alleles in expression levels, rather than the all-or-none qualitative differences observed at imprinted autosomal loci (Haig 2000a).

The argument for the evolutionary instability of pXCI is a two-edged sword. It provides an explanation for the origin of rXCI from pXCI in an ancestor of eutherian mammals, but does not provide a compelling explanation for why pXCI is maintained in marsupials and murine trophoblast. In the case of marsupials, one might appeal to

phylogenetic inertia and the difficulty of evolving rXCI. Lee (2003a) has argued that pXCI in marsupials, unlike rXCI, does not require a counting mechanism to ensure that one, and only one, X chromosome is active in each cell. Perhaps coordination between homologs to determine which remains active is the "high hurdle" to the origin of rXCI that explains the persistence of pXCI in marsupials. But this argument does not help in the case of murine trophoblast because mechanisms of rXCI exist in somatic cells of mice and rXCI has been described from human trophoblast (Willemsen et al. 2002; Zeng and Yankowitz 2003). I will argue below that pXCI in trophoblast, unlike pXCI in marsupials, may have evolved from rXCI.

rXCI as a competitive process

Once rXCI is established and has an obligate role in dosage regulation, the evolution of locus-by-locus imprinting on the X chromosome creates a competitive dynamic with respect to the choice of which X chromosome remains active. X^P will be less inhibitory than X^M in its effects on demand. Therefore, the two X chromosomes should compete to be the one that remains active, particularly in tissues that mediate demand. If so, pXCI in mouse trophoblast, unlike pXCI in marsupials, may have evolved *from* rXCI and involved X^M forcing its less inhibitory homolog to shut down. Such a form of "enforced" pXCI is evolutionarily unstable in the short and long-term. In the short-term there is selection on the padumnal X to resist inactivation. In the long-term, if the madumnal X wins out, X-linked genes cease to be selected when paternally derived, thus favoring a reversion to rXCI (see previous section). Two factors possibly constrain competition to inactivate one's homolog. First, marginal increases or decreases in demand (as would

result from small biases in inactivation) will, respectively, benefit patrilineal and matrilineal interests. However, large biases might cause large changes in demand that are in neither chromosome's interests. Second, the greater the bias in inactivation, the closer the approach to functional hemizygosity with its associated costs.

pXCI has been reported from preimplantation mouse embryos (Huynh and Lee 2003; Mak et al. 2004; Okamoto et al. 2004) and extraembryonic tissues of cattle (Xue et al. 2002), rats (Wake et al. 1976), and mice (Takagi and Sasaki 1975), as well as humans (Harrison 1989). Recent data, however, suggest that XCI in human trophoblast is "random" (Willemsen et al. 2002; Zeng and Yankowitz 2003). There may also be exceptions to pXCI in mouse trophoblast. Huynh and Lee (2001) have hypothesized that pXCI in mouse trophoblast is "leaky" and that a subset of cells may inactivate the madumnal rather than the padumnal X. Hadjantonakis et al. (2001) have reported that both X chromosomes are active in trophoblast giant cells of mice. If rXCI is a competitive process in which different chromosomes vie to remain active, the observed cases of pXCI in trophoblast may simply lie at one end of a continuum of progressively greater skew as to which chromosome is inactivated. The difference between pXCI in mouse trophoblast and rXCI in human trophoblast would then be quantitative rather than qualitative.

The mechanisms that determine which X chromosome is to be inactivated in murine trophoblast are still poorly understood, but some data are compatible with a model in which X^M "refuses" to shut down, thus forcing the inactivation of X^P. For example, neither copy of X^M is inactivated in X^MX^MY and $X^MX^MX^P$ blastocysts—in the latter case X^P is inactivated—but rXCI is reported in X^PX^P androgenetic embryos (Goto and Takagi

2000; Okamoto et al. 2000). This could be viewed as the outcome of an evolutionary process in which X^M has gained the upper hand in competition with X^P to remain active in trophoblast.

Evidence to test the hypothesis that X^M and X^P are in competition to remain active in somatic cells may come from an understanding of the mechanisms of skewed X-inactivation caused by natural genetic variation at the X-inactivation center. Such variation has been reported in mice (Cattanach 1975) and humans (Plenge et al. 1997; Pugacheva et al. 2005). Falconer and colleagues (Falconer and Isaacson 1972; Falconer et al. 1982) were able to select for increased and decreased expression of the *brindled* phenotype in mice (a phenotype due to 'random' inactivation of alleles at an X-linked coat color locus). The response to selection could be due either to skewed XCI or to differential survival of cells with different active alleles. Of particular interest, the proportion of cells expressing the *brindled* allele in heterozygous females depended on parental origin, suggesting an imprinting effect.

The *Tsix* transcript (antisense to *Xist*) of mice clearly plays an important role in pXCI in trophoblast and rXCI in somatic cells (Lee 2000). However, it is currently controversial whether this role is conserved in humans (Migeon 2003; Lee 2003b). A lack of conservation of basic mechanisms of XCI would be compatible with a model in which there is ongoing conflict over which X remains active.

PAM and its precursors

Previous sections have presented a model of the origin of XCI that I will call the parental antagonism model (PAM). PAM proposes that pXCI preceded rXCI and that

pXCI evolved because of intragenomic conflicts between madumnal and padumnal alleles. PAM further proposes that XCI, once established, is self-reinforcing because it acquires a role in dosage regulation, but that pXCI is evolutionarily unstable because of the inherent advantages of rXCI. Once rXCI is established, however, imprinting of X-linked loci leads to competition between X^M and X^P to remain active.

PAM is not the first model to attempt to explain the evolution of pXCI in terms of the demands that mammalian offspring make on their mothers. Moore and Haig (1991) argued that equalization of gene dosage between the sexes requires pXCI if there is selection for genomic imprinting, because then both sexes have a single active madumnal X chromosome. Up-regulation of the single X in males, as occurs in *Drosophila*, would not equalize expression between the sexes if X-linked genes are imprinted because males lack a padumnal X chromosome. In our view, rXCI was favored over pXCI in tissues where there was relatively weak selection for imprinting; pXCI was seen as being imposed on X^P whereas rXCI was self-enforced. My implicit assumption, at the time, was that X^P expressed demand enhancers whose expression was suppressed by other elements of the genome. My current views, as expressed in PAM, differ from this earlier model in two important respects. First, the X chromosome carries demand inhibitors rather than enhancers. Second, PAM does not invoke a need to equalize dosage between the sexes. I consider the earlier model to be superseded by PAM.

In a subsequent paper, Moore et al. (1995) proposed that the presence of a double dose of X-linked demand *enhancers* in females caused female offspring to receive more resources than male offspring (*contra* PAM, which invokes X-linked demand *inhibitors*). The resulting sex-ratio distortion created the selective force favoring reduced expression

of demand enhancers in female embryos. This was achieved by the inactivation of X^P after fertilization by maternally-encoded modifiers acting in *trans*. Moore et al. (1995) do not provide any clear justification of why it should be X^P that is inactivated because their assumption that demand enhancers were expressed in double dose in females implies that the two X chromosomes were functionally interchangeable. Although I consider that Moore et al. (1995) have provided an inadequate explanation for the origin of XCI, parts of their paper are strong. In particular, they argue that the origin of pXCI caused X-linked genes to be subject to selection only on their effects when maternally derived and that this favored replacement of pXCI by rXCI. This anticipates arguments used in PAM.

Reik and Lewis (2005) have recently proposed that pXCI replaced an ancestral mechanism of dosage compensation because of selection to reduce the padumnal expression of X-linked growth suppressors. This scenario is clearly similar to PAM, although the authors provide no detailed discussion of the selective forces involved, including no explanation as to why the X chromosome should carry growth suppressors rather than growth enhancers.

Comparisons with the dosage-compensation model

The standard model for the evolution of XCI invokes a need for dosage compensation brought about by the degeneration of genes on the mammalian Y chromosome (Charlesworth 1978, 1996; Jegalian and Page 1998). In this model, the degeneration of Y-linked genes (step 1) favored increased expression of their X-linked homologs (step 2) because of benefits to males. Increased expression in males was associated with a correlated, but maladaptive, increase of expression in females that created a selective

force favoring the sex-limited down-regulation of X-linked genes in females (step 3). In mammals, this down-regulation was achieved by silencing one of the two X chromosomes in females. I will call this the sexual-antagonism model (SAM) of XCI because the up-regulation of X-linked expression in step 2 is proposed to have been beneficial to males but costly to females.

The quantitative relations between benefits and costs do not appear to have been explicitly stated in any of the verbal formulations of SAM. In fact, there has been a remarkable absence of any formal development of SAM. I will not attempt such an analysis here, but will simply point out some unstated implications of SAM. Suppose that fitness w was a continuous function of z, the total level of expression of some factor with X-linked and Y-linked alleles

$$w = f(z) \tag{1}$$

Further suppose that the fitness of both sexes was maximized by a total level of expression z^* toward which X-linked and Y-linked alleles (call these alleles X_1 and Y_1) each contributed $z^*/2$ prior to inactivation of the Y-linked allele.

An inactivating mutation of the Y-linked allele, Y_{null}, would have caused X_1Y_{null} males to express $z^*/2$ rather than z^*. Therefore, the difference in fitness between an X_1Y_{null} male and an X_1Y_1 male would have been

$$f\left(\frac{z^*}{2}\right) - f(z^*) = \Delta w_m < 0 \tag{2}$$

Here, Δw_m is a measure of the fitness hurdle that had to be overcome by genetic drift or hitch-hiking for Y_{null} to become established in the population (step 1).

Now suppose that a *cis*-acting mutation, X_2, caused a doubling of expression of the factor from its X-linked allele such that X_2Y_{null} males now expressed z^* whereas X_1X_2 females expressed $3z^*/2$. Such a mutation would have had sexually-antagonistic effects: X_2Y_{null} males would be more fit than X_1Y_{null} males but X_1X_2 females would be less fit than X_1X_1 females. The difference in fitness between an X_2Y_{null} male and an X_1Y_{null} male would have been

$$f(z^*) - f\left(\frac{z^*}{2}\right) = -\Delta w_m > 0 \tag{3}$$

Thus, the substitution of X_2 for X_1 reverses the effect of the substitution of Y_{null} for Y_1 (in males). However, the difference in fitness between between an X_1X_2 female and an X_1X_1 female would have been

$$f\left(\frac{3z^*}{2}\right) - f(z^*) = \Delta w_f < 0 \tag{4}$$

The new mutation, X_2, would have been expressed twice as frequently in females as in males. Therefore, X_2 would have increased in frequency by natural selection (step 2) if

$$2\Delta w_f - \Delta w_m > 0 \tag{5}$$

As X_2 increased in frequency, some females would have received two copies and expressed $2z^*$. Clearly, whether X_2 could have spread to fixation would have depended on the precise form of $f(z)$. Inequality (5) implies that fitness must have declined more rapidly below, than above, the optimum expression level at z^*. However, the more rapidly fitness declined below z^*, the higher would have been the fitness hurdle that had to be overcome by the initial inactivating mutation Y_{null}.

If X_2 became established in a population, then females would have benefited from the origin of an inactivatable allele X_3 that restored expression levels to z^* in both sexes (step 3). However, a fitness function that is relatively insensitive to expression above z^* implies that increased expression in females would be only weakly deleterious, so that the compensatory down-regulation of X-linked expression in females would be only weakly selected. It could be argued that "X_3" reduced expression in females at many X-linked loci with sexually antagonistic effects, such that the fitness effect of reducing expression at one locus was weak but the cumulative advantage of halving expression at all loci was strong. Yet, the invocation of multiple loci raises the question why the "X_2" mutations at multiple X-linked loci should all have caused a roughly two-fold increase in expression such that a 50% reduction in expression at each locus is advantageous. (Why wouldn't some of the "X_2" mutations have increased expression by 20%, others by 40%, and so on?) These heuristic arguments do not prove that SAM cannot explain the origin of XCI, but they do suggest that the conditions for such a model to work are more constrained than has been tacitly assumed in previous verbal presentations of SAM.

SAM has nothing to say about why dosage compensation in mammals was achieved by silencing one of the two X chromosomes of females, rather than by a symmetrical

down-regulation of both X chromosomes (as occurs in *Caenorhabditis*; Kelley and Kuroda 1995). Under SAM, the mechanism of dosage compensation employed by a particular taxon is purely arbitrary. I have argued above that XCI is a curious means of achieving dosage compensation because it forgoes some of the benefits of diploidy and requires that identical sequences be expressed differently in a single nucleus. In mitigation of this last criticism, a number of writers have suggested that pXCI preceded rXCI and simply required that the inactivation of the X chromosome that occurs during normal male meiosis be maintained into the next generation (Lifschytz and Lindsley 1972; Lyon 1974; Huynh and Lee 2001, 2005; Wu and Xu 2003).

SAM argues that XCI evolved because of conflicting selective forces acting on expression level per allele in males and females. By contrast, PAM argues that X^M and X^P evolved differential expression because they were subject to divergent selective forces *within females*. That is, X^M was subject to selection for increased expression of demand inhibitors, whereas X^P was subject to selection for decreased expression. Therefore, differential expression of homologs is an intrinsic feature of PAM, rather than a special assumption of SAM. PAM was developed explicitly to address mammalian XCI whereas SAM was initially presented as a brief addendum to a model of the degeneration of the Y chromosome and the evolution of dosage compensation that was intended to apply to multiple origins of these phenomena in diverse taxa (Charlesworth 1978). PAM does not contest this broader model of the degeneration of the Y chromosome, but only its application to the evolution of XCI in an ancestral mammal.

To reiterate features of the current model, PAM assumes that both X-linked alleles were active in an ancestral mammal prior to the origin of pXCI but is neutral about

whether this ancestor already possessed a mechanism of dosage compensation that did not involve XCI; PAM is also neutral on the presence or absence in this ancestor of functional homologs of X-linked alleles on the Y chromosome; PAM accepts that XCI now performs an essential role in dosage regulation that is important for the *maintenance* of XCI. PAM does not invoke a need to equalize expression between the sexes, although this could be a factor favoring the spread of inactivation to loci not involved in the inhibition of demand.

SEXUAL VS. PARENTAL ANTAGONISM ON THE X CHROMOSOME

Sexual and parental antagonism are two sides of the same coin. A gene that is present in a male body in this generation is necessarily paternally-derived in the next generation, whereas a gene that is present in a female body in this generation is necessarily maternally-derived in the next. On the other hand, whether an allele at an autosomal locus is maternally or paternally derived provides no information about its associated sex in the current generation (or its parental origin and associated sex in the next generation). This is because both males and females have a madumnal and padumnal allele at each autosomal locus, and these alleles are distributed to offspring independently of the offspring's sex. By contrast, parental origin provides information about current and future sex at X-linked loci because only females possess padumnal alleles. Therefore, a padumnal allele is necessarily present in a female body, and a madumnal allele is more likely to be transmitted to a female body than to a male body in the next generation. Moreover, a gene with male-limited expression is only expressed when maternally-derived, whereas a gene that is only expressed when paternally-derived has female-

limited expression. Thus, questions of sexual and parental antagonism are entangled at X-linked loci.

The effects of X-linked genes are predicted to be biased in favor of females (because an X-linked allele has spent two-thirds of its ancestry in females) and biased in favor of matrilines (because an X-linked allele has been transmitted by eggs twice as often as by sperm). By contrast, the average fitness effects of autosomal genes are predicted to be unbiased with respect to sex and parental origin. The relative importance of these two X-linked biases is currently unclear. For example, there is purported to be an excess of genes promoting general intelligence on the human X chromosome (Lehrke 1972; Turner 1996). An interesting evolutionary question arises if this excess is not simply an artifact of a greater ease of ascertainment for X-linked loci. Is the X chromosome disproportionately involved in the control of intelligence because, during the course of human evolution, incremental increases of intelligence have been of greater value to women than to men (sexual antagonism) or because they have been of greater value to matrilines than to patrilines (parental antagonism)?

A recurrent suggestion has been that imprinting of X-linked loci may have a role in the development of sexual dimorphism. This has been suggested for marsupials (Cooper et al. 1993; Graves 1996), for humans (Skuse 1999), and for the entirely X-linked genomes of haplodiploid insects (Poirié et al. 1992). Iwasa and Pomiankowski (1999, 2001) have argued that theoretical models and the available data are more consistent with imprinting of X-linked loci having evolved to achieve sexual dimorphism rather than to mediate evolutionary conflicts between madumnal and padumnal alleles. I believe this conclusion is premature: sexual and parental antagonism *both* need to be considered in

models of X-linked imprinting. If female-limited expression is achieved by inactivating an allele when maternally-derived, the allele will thereby only be subject to selection for its effects on matrilineal inclusive fitness. One might ask however why female-limited expression should be achieved indirectly by inactivating madumnal alleles in both males and females, rather than directly by inactivating the single allele in males (good old-fashioned unimprinted sex limitation). The model of Iwasa and Pomiankowski (2001) begs the question by assuming that imprinting is the only way of achieving sex-specific expression.

A comparison between the phenotypic effects of genes on marsupial and eutherian X chromosomes has some potential to separate the effects of parental and sexual antagonism. This is because pXCI eliminates the predicted female bias in the effects of X-linked genes but accentuates the matrilineal bias. The matrilineal bias of X-linked genes is accentuated in marsupials because genes subject to pXCI are never exposed to selection for their effects when paternally derived (Haig 2000b). The female bias in the effects of X-linked genes is eliminated because madumnal X-linked alleles are equally likely to be present in male and female bodies.

OVERVIEW

Over the course of evolutionary time, some allelic substitutions at autosomal loci will have been associated with inclusive fitness benefits when paternally-derived that outweighed inclusive fitness costs when maternally-derived, whereas others will have been associated with the opposite pattern of antagonistic effects. As a result, autosomal loci as a class are not predicted to systematically favor matrilines or patrilines, and loci

whose expression favors patrilines are likely to be intermingled with loci whose expression favors matrilines on individual autosomes. Therefore, autosomal imprinting is predicted to evolve locus-by-locus rather than involving the inactivation of entire chromosomes. Allelic substitutions at unimprinted X-linked loci however are predicted to have been biased in favor of matrilines. At matrilineally-biased loci with dosage-sensitive effects, natural selection favors the silencing of padumnal alleles. Therefore, parental antagonism is proposed to have provided an evolutionary force that favored the inactivation of the padumnal X chromosome (pXCI) in females of an ancestral mammal.

Once pXCI had evolved, natural selection would have favored increased expression of genes on the madumnal X chromosome to reassert matrilineal interests. As a consequence, the activity of two X chromosomes came to be incompatible with successful development, and X chromosome inactivation had acquired an essential role in 'dosage compensation.' However, the disadvantages of functional hemizygosity favored the replacement of pXCI by random X chromosome inactivation (rXCI).

By this hypothesis, genomic imprinting and XCI have evolved in mammals because of genes that have asymmetric effects for matrilines and patrilines. In mammals, the symmetry of effects is broken by lactation, viviparity, and sex-biased dispersal (Haig 2000b), with the costs of parental care being borne disproportionately by matrilines (in particular, by mothers). Asymmetric effects are absent when genetic actions affect only the individual in which a gene is expressed and the individual's direct descendants, plus other individuals that are randomly selected from the population without regard to relatedness. These conditions are approximated by oviparous organisms without postzygotic parental care (such as *Drosophila* and *Caenorhabditis*). Therefore, significant

effects of imprinting are not expected in such organisms, and dosage compensation (if it occurs) should involve mechanisms other than XCI.

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