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SEXUAL ANTAGONISM AND THE EVOLUTION OF X CHROMOSOME INACTIVATION

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In most female mammals, one of the two X chromosomes is inactivated early in embryogenesis. Expression of most genes on this chromosome is shut down, and the inactive state is maintained throughout life in all somatic cells. It is generally believed that X-inactivation evolved as a means of achieving equal gene expression in males and females (dosage compensation). Following degeneration of genes on the Y chromosome, gene expression on X chromosomes in males and females is upregulated. This results in closer to optimal gene expression in males, but deleterious overexpression in females. In response, selection is proposed to favor inactivation of one of the X chromosomes in females, restoring optimal gene expression. Here, we make a first attempt at shedding light on this intricate process from a population genetic perspective, elucidating the sexually antagonistic selective forces involved. We derive conditions for the process to work and analyze evolutionary stability of the system. The implications of our results are discussed in the light of empirical findings and a recently proposed alternative hypothesis for the evolution of X-inactivation.

KEY WORDS: **Dosage compensation, intralocus sexual conflict, mammals, model, sex chromosome evolution, sexual antagonism.**

Ohno et al. (1959) showed that only one of the two X chromosomes of female rats formed the sex chromatin in nuclei of liver cells. They tentatively suggested that the heteropycnotic chromosome was the paternally derived X chromosome because the maternally derived X chromosome of male cells was not condensed. However, Ohno and Hauschka (1960) soon favored a theory in which heteropycnosis alternated between the two X chromosomes.

In a brief letter to *Nature*, Lyon (1961) proposed that the dappled phenotype of female mice heterozygous for X-linked coat color mutants could be explained by "inactivation of one or other X-chromosome *early in embryonic development*" (emphasis added). Soon after, Beutler et al. (1962) reported studies of women heterozygous for X-linked glucose-6-phosphate dehydrogenase (G6PDH) deficiency. The blood of these women contained two populations of erythrocytes, one population possessing, and the other lacking, G6PDH activity. They wrote, "It seems necessary, therefore, to assume that there is, at least for some period of time during development, randomization of the active and inactive chromosome among the dividing cells of the body. Women would then be a functional mosaic of cells, some with a functional paternal X-chromosome, others with a functional maternal X-chromosome." In a longer exposition of her hypothesis, Lyon (1962) wrote that the "cytologic evidence suggests the inactivation of one X is the typical method of *dosage compensation* in female mammals" (emphasis added). Ever since, it has generally been assumed that equalization of gene dosage between males and females is the reason for the evolution of X chromosome inactivation (XCI) in mammals. The idea is so intuitively attractive that it has been subject to little formal evolutionary analysis.

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Charlesworth (1978, 1996) presented a verbal sketch of what has become the standard model for the evolution of XCI. The model starts with an X and Y chromosome that share the same gene loci, but do not recombine. In the first step, genes on the Y chromosome degenerate, for example by the operation of Muller's ratchet or due to background selection. This creates an inequality of dosage between males with a single X chromosome and females with two X chromosomes, in which the overall level of expression is assumed to be optimal in females but suboptimal in males. In the second step, natural selection on males favors the upregulation of X-linked loci; this upregulation is manifested in both sexes, causing an increase in male fitness but a decrease in female fitness. In the final step, natural selection on females favors the inactivation of one of the X chromosomes to equalize dosage between males and females. Haig (2006) called this the sexual antagonism model (SAM) for the evolution of XCI because the second step was beneficial for males but costly for females. Empirical evidence for SAM comes from the observation that in several mammals, genes on the active X chromosome are expressed at about twice the level as autosomal genes (Nguyen and Disteche 2006). However, this observation is subject to alternative explanations: e.g., upregulation of a single X chromosome may have been an older mechanism of dosage compensation (resembling that in *Drosophila*) that was supplanted by XCI.

Step 1 of this process—the degeneration of the Y chromosome—has been studied both empirically and theoretically and is not confined to mammals (reviewed in Charlesworth and Charlesworth 2000; Bachtrog 2006). Surprisingly, steps 2 and 3 of SAM appear never to have been formally modelled. In this article, we present a first attempt at such a model.

The Model

We assume an infinitely large, randomly mating population of XX females and XY males with discrete and nonoverlapping generations. We consider an X-linked locus **M** that is under selection and assume that the corresponding locus on the Y chromosome is completely nonfunctional. An individual's fitness depends on the total amount of gene product (z) expressed from his or her X-linked allele(s). The fitness function $w(z)$ is assumed to be differentiable and unimodal with a maximum at $w(2) = 1$, and to be identical for males and females. Gene expression at the focal locus is assumed to be regulated in *cis*. We consider two alleles, m and M. Each copy of allele m contributes to the level of gene expression by an amount $z = 1$ in both sexes, whereas each copy of allele M contributes to expression by an amount $z = 1 + c$ in males and amount $z = 1 + \alpha c$ in females ($0 < c \leq 1$; $0 \leq \alpha \leq 1$). We further assume that the population is initially fixed for allele m such that mm females produce $z = 2$ units of gene product but m/Y males produce $z = 1$. Thus, the overall level of expression is optimal in females, $w(2) = 1$, but suboptimal in males, $w(1) < 1$. The allele M is introduced to the population to model the effect of upregulation of the gene in males (with a pleiotropic upregulation in females). The parameter α is a measure of the degree of sex-specificity of this upregulation.

A second X-linked locus **I** is introduced to emulate XCI. The ancestral allele at this locus (i) does not affect expression at **M**, whereas the mutant allele I shuts down the linked allele at the **M** locus in females. We make two assumptions concerning the action of I. First, inactivation occurs in *cis*, so that it affects only the allele at **M** on the same chromosome. Second, in II females only one, randomly chosen allele at **M** is inactivated (see Discussion). The **M** and **I** loci recombine with rate r ($0 \leq r \leq 1/2$) in females.

We denote by x_{jk} and y_{jk} the frequencies of the different genotypes of eggs and sperm, respectively. Here, j ($j = m$ or M) represents the **M** locus and k ($k = i$ or I) represents the **I** locus. There are 10 possible genotypes in females and four in males (Table 1). Figure 1 illustrates the fitness function $w(z)$, including fitness values for all possible genotypes. Note that although these values may vary according to the shape of w, the order of the

Table 1. Possible genotypes in males and females, their frequency at zygote stage depending on gamete frequencies, and their fitness.

Females			Males		
Genotype	Frequency	Fitness	Genotype	Frequency	Fitness
mi/mi	$x_{mi}y_{mi}$	$w(2)$	*mi*	x_{mi}	$w(1)$
Mi/mi	$x_{Mi}y_{mi}+x_{mi}y_{Mi}$	$w(2+\alpha c)$	*Mi*	x_{Mi}	$w(1+c)$
Mi/Mi	$x_{Mi}y_{Mi}$	$w(2+2\,\alpha c)$	*mI*	x_{mI}	$w(1)$
mI/mi	$x_{mI}y_{mi}+x_{mi}y_{mI}$	$w(1)$	*MI*	x_{MI}	$w(1+c)$
MI/mi	$x_{MI}y_{mi}+x_{mi}y_{MI}$	$w(1)$			
Mi/mI	$x_{Mi}y_{mI}+x_{mI}y_{Mi}$	$w(1+\alpha c)$			
MI/Mi	$x_{MI}y_{Mi}+x_{Mi}y_{MI}$	$w(1+\alpha c)$			
mI/mI	$x_{mI}y_{mI}$	$w(1)$			
MI/mI	$x_{MI}y_{mI}+x_{mI}y_{MI}$	$w(1+\alpha c/2)$			
MI/MI	$x_{MI}y_{MI}$	$w(1+\alpha c)$			

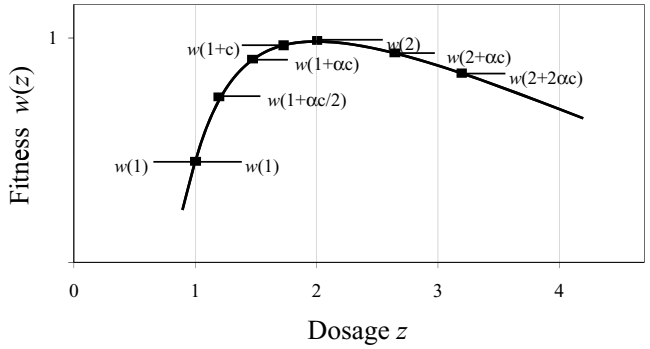

Figure 1. Illustration of the fitness function w depending on dosage z. The only assumptions that we make about the shape of $w(z)$ are differentiability and unimodality with the maximum at $w(2) = 1$. Also shown are fitness values for all possible genotypes in the population. Captions for fitness values for males are shown on the left, and for females to the right, of the curve.

fitness values on either side of $z = 2$ is strictly as shown in Figure 1.

From the genotype frequencies in Table 1, the following recursion equations for the gamete frequencies can be obtained:

$$\begin{aligned}
\bar{W}_x x'_{mi} &= 2x_{mi}y_{mi}w(2) + (x_{Mi}y_{mi} + x_{mi}y_{Mi})w(2+\alpha c) \\
&\quad + (x_{mI}y_{mi} + x_{mi}y_{mI})w(1) \\
&\quad + (1-r)(x_{MI}y_{mi} + x_{mi}y_{MI})w(1) \\
&\quad + r(x_{Mi}y_{mI} + x_{mI}y_{Mi})w(1+\alpha c) \\
\bar{W}_x x'_{Mi} &= (x_{Mi}y_{mi} + x_{mi}y_{Mi})w(2+\alpha c) + 2x_{Mi}y_{Mi}w(2+2\alpha c) \\
&\quad + r(x_{MI}y_{mi} + x_{mi}y_{MI})w(1) \\
&\quad + (x_{MI}y_{Mi} + x_{Mi}y_{MI})w(1+\alpha c) \\
&\quad + (1-r)(x_{Mi}y_{mI} + x_{mI}y_{Mi})w(1+\alpha c) \\
\bar{W}_x x'_{mI} &= (x_{mI}y_{mi} + x_{mi}y_{mI})w(1) \\
&\quad + r(x_{MI}y_{mi} + x_{mi}y_{MI})w(1) \\
&\quad + (1-r)(x_{Mi}y_{mI} + x_{mI}y_{Mi})w(1+\alpha c) \\
&\quad + 2x_{mI}y_{mI}w(1) + (x_{MI}y_{mI} + x_{mI}y_{MI})w(1+\alpha c/2) \\
\bar{W}_x x'_{MI} &= (1-r)(x_{MI}y_{mi} + x_{mi}y_{MI})w(1) \\
&\quad + (x_{MI}y_{Mi} + x_{Mi}y_{MI})w(1+\alpha c) \\
&\quad r(x_{Mi}y_{mI} + x_{mI}y_{Mi})w(1+\alpha c) \\
&\quad + (x_{MI}y_{mI} + x_{mI}y_{MI})w(1+\alpha c/2) + 2x_{MI}y_{MI}w(1+\alpha c) \\
\bar{W}_y y'_{mi} &= x_{mi}w(1) \\
\bar{W}_y y'_{Mi} &= x_{Mi}w(1+c) \\
\bar{W}_y y'_{mI} &= x_{mI}w(1) \\
\bar{W}_y y'_{MI} &= x_{MI}w(1+c)
\end{aligned} \tag{1}$$

In these equations, $\bar{W}_x$ and $\bar{W}_y$ are given by the sum of all terms on the right side of the first four and last four equations, respectively.

Results

INVASION OF UPREGULATING ALLELE

If the inactivating allele I is not present in the population, then our model reduces to a model with sexually antagonistic fitness effects at a single locus, similar to previous models (e.g., Haldane and Jayakar 1964). The fitness of m/Y males is $w(1)$ and the fitness of M/Y males is $w(1 + c)$, where $w(1 + c) > w(1)$. The respective fitnesses of mm, Mm, and MM females are $w(2)$, $w(2 + \alpha c)$, and $w(2 + 2\,\alpha c)$, where $w(2) \geq w(2 + \alpha c) \geq w(2 + 2\,\alpha c)$. Thus, invasion of M increases male fitness but reduces female fitness if $\alpha > 0$ (see Fig. 1).

Recursion equations (1) can be simplified to

$$\begin{aligned}
x'_M = {} & [(x_M + y_M - 2x_M y_M)w(2+\alpha c) + 2x_M y_M w(2+2\alpha c)]/ \\
& [2(1-x_M)(1-y_M)w(2) + 2(x_M + y_M - 2x_M y_M)w(2+\alpha c) \\
& + 2x_M y_M w(2+2\alpha c)],
\end{aligned} \tag{2a}$$

$$y'_M = \frac{x_M w(1+c)}{(1-x_M)w(1) + x_M w(1+c)}. \tag{2b}$$

In these equations, we have omitted subscripts for the **I** locus and exploited the fact that $x_m = 1 - x_M$ and $y_m = 1 - y_M$. Aside from the two "trivial" fixed points corresponding to fixation of m and M, this simplified system may have one polymorphic fixed point, given by

$$\begin{aligned}
&(\hat{x}_M, \hat{y}_M) = \\
&\left(\frac{2w(1)w(2) - (w(1) + w(1+c))w(2+\alpha c)}{2[w(1)w(2) + w(1+c)w(2+2\alpha c) - (w(1) + w(1+c))w(2+\alpha c)]}, \right. \\
&\left. \frac{w(1+c)w(2+\alpha c)(w(1) + w(1+c)) - 2w(1)w(1+c)w(2)}{w(2+\alpha c)[w(1) + w(1+c)]^2 - 2w(1)w(1+c)[w(2) + w(2+2\alpha c)]} \right).
\end{aligned} \tag{3}$$

Stability of the fixed points can be determined by analyzing the Jacobian matrix of the dynamical system. Evaluated at $(x_M, y_M) = (0, 0)$, the Jacobian matrix is

$$\mathbf{J}|_{(x_M, y_M)=(0,0)} = \begin{pmatrix} \dfrac{w(2+\alpha c)}{2w(2)} & \dfrac{w(1+c)}{w(1)} \\ \dfrac{w(2+\alpha c)}{2w(2)} & 0 \end{pmatrix},$$

with leading eigenvalue

$$\lambda = \frac{w(2+\alpha c) + \sqrt{[8w(2+\alpha c)w(1+c)w(2) + w(1)w(2+\alpha c)^2]/w(1)}}{4w(2)}.$$

Therefore, the condition for M to spread in the population when rare ($\lambda > 1$) is

$$\frac{w(1+c) - w(1)}{w(1)} > 2\left\{ \frac{w(2) - w(2+\alpha c)}{w(2+\alpha c)} \right\}. \tag{4}$$

Condition (4) differs from, and corrects, condition (5) of Haig (2006). The latter is a good approximation for small fitness differences. Provided it can invade, M will spread to fixation if

$$\frac{w(1+c)-w(1)}{w(1+c)} > 2\left\{\frac{w(2+\alpha c)-w(2+2\alpha c)}{w(2+\alpha c)}\right\} \quad (5)$$

Both alleles will be maintained at the polymorphic equilibrium given in (3) if condition (4), but not (5), is satisfied. Note that because of sexually antagonistic selection a polymorphic equilibrium is possible even with $w(2) \geq w(2+\alpha c) \geq w(2+2\,\alpha c)$ as assumed in the model (i.e., no overdominance).

EVOLUTIONARY DYNAMICS OF X CHROMOSOME UPREGULATION

Although our model includes only two upregulating alleles, the results of the previous section can be generalized to predict the long-term evolutionary behavior of the system, assuming the availability of the respective genetic variation at the **M** locus and no XCI. Let us consider two upregulating alleles M_1 and M_2, with parameters (c_1, α_1) and (c_2, α_2), respectively. From inequality (4), it is clear that a population fixed for M_1 is stable against invasion of M_2 if

$$\frac{w(1+c_2)-w(1+c_1)}{w(1+c_1)} \leq 2\left\{\frac{w(2+2\alpha_1 c_1)-w(2+\alpha_1 c_1+\alpha_2 c_2)}{w(2+\alpha_1 c_1+\alpha_2 c_2)}\right\}. \quad (6)$$

For $c_1 = c_2$, this inequality is equivalent to $w(2+(\alpha_1+\alpha_2)c_1) \leq w(2+2\,\alpha_1\,c_1)$. This demonstrates that in a population in which M_1 is fixed, any allele M_2 with $c_2 = c_1$, but $\alpha_2 < \alpha_1$ can invade. This makes intuitive sense because smaller values of α result in reduced deleterious overexpression of the allele in females. An evolutionarily stable allele $\hat{M}$ therefore is characterized by $\hat{\alpha} = 0$. This in turn implies $\hat{c} = 1$, because with $\hat{\alpha} = 0$ only selection on optimal dosage in males is relevant (inequality (6) simplifies to $w(1+c_2) \leq w(1+c_1)$). In summary, the only evolutionarily stable allele $\hat{M}$ is characterized by the parameters $\hat{c} = 1$ and $\hat{\alpha} = 0$ (male-limited, twofold upregulation). This resembles the mechanism of dosage compensation observed in *Drosophila melanogaster*. Clearly, there would be no evolutionary advantage of XCI if all X-linked loci employed this mode of dosage compensation.

For XCI to evolve, an inactivating allele must invade before fixation of $\hat{M}$ or there must be a constraint on sex-specific expression of M. We will therefore now assume that α takes a fixed value, and consider an allele $\tilde{M}$ with parameters $(\tilde{c},\ \alpha)$. We analyze the evolutionary stability of this allele against invasion of an allele with $(\tilde{c}+\varepsilon,\ \alpha)$, where ε is very small. From inequality (6), $\tilde{M}$ is stable if

$$\frac{w(1+\tilde{c}+\varepsilon)-w(1+\tilde{c})}{w(1+\tilde{c})} \leq 2\left\{\frac{w(2+2\alpha\tilde{c})-w(2+2\alpha\tilde{c}+\alpha\varepsilon)}{w(2+2\alpha\tilde{c}+\alpha\varepsilon)}\right\} \quad (7)$$

is true for all sufficiently small ε. Linearization of the fitness function w leads to

$$\frac{[w(1+\tilde{c})+\varepsilon w'(1+\tilde{c})]-w(1+\tilde{c})}{w(1+\tilde{c})} \leq 2\left\{\frac{w(2+2\alpha\tilde{c})-[w(2+2\alpha\tilde{c})+\alpha\,\varepsilon\,w'(2+2\alpha\tilde{c})]}{w(2+2\alpha\tilde{c})+\alpha\,\varepsilon\,w'(2+2\alpha\tilde{c})}\right\}, \quad (8)$$

which, after ignoring terms in ε^2, is equivalent to

$$\varepsilon\frac{w'(1+\tilde{c})}{w(1+\tilde{c})} \leq -2\alpha\varepsilon\frac{w'(2+2\alpha\tilde{c})}{w(2+2\alpha\tilde{c})}. \quad (9)$$

Thus, $\tilde{M}$ is stable if

$$\frac{w'(1+\tilde{c})}{w(1+\tilde{c})} = -2\alpha\frac{w'(2+2\alpha\tilde{c})}{w(2+2\alpha\tilde{c})}. \quad (10)$$

There must be at least one solution $\tilde{c}$ to equation (10) because the left-hand side is zero if $\tilde{c} = 1$, the right-hand side is zero if $\tilde{c} = 0$, and both sides are nonnegative for all $0 \leq \tilde{c} \leq 1$. (Note that there is exactly one solution $\tilde{c}$ with $0 < \tilde{c} \leq 1$ if w is concave). For $\alpha = 0$, $\tilde{c} = 1$ (expression is optimal in both sexes). For $\alpha > 0$, $\tilde{c} < 1$ (expression is suboptimal in males and superoptimal in females, with greater departures from optimality as α increases). If dosage affects fitness only weakly (i.e., $w(z) \approx 1$), condition (10) can be approximated by $w'(1+\tilde{c}) \approx -2\alpha w'(2+2\alpha\tilde{c})$. This can be interpreted such that at equilibrium upregulation $\tilde{c}$, the ratio between fitness increase in males and fitness decrease in females with increasing dosage z is given by 2α.

INVASION OF INACTIVATING ALLELE

Three circumstances can be distinguished in which the I (inactivation) allele arises by mutation. First, if the population is fixed for m, then I is selected against and cannot invade because $w(1) < w(2)$. Second, if the population is fixed for M (including the evolutionarily stable allele $\tilde{M}$), then *Ii* females have fitness $w(1+\alpha c)$ compared to $w(2+2\,\alpha c)$ for *ii* females. Because I is without effect in males (by assumption of the model), I will invade and spread to fixation if

$$w(1+\alpha c) > w(2+2\alpha c). \quad (11)$$

Both of these rather intuitive results are confirmed by formal eigenvalue analysis of the Jacobian matrix of the full dynamical system given in (1) (analysis not shown). When upregulation is sex-specific to some extent ($\alpha < 1$), condition (11) tends to be fulfilled when overexpression is deleterious (small $w(2+2\,\alpha c)$), but underexpression is not (large $w(1+\alpha c)$). This is in direct contrast to the conditions that favor spread and fixation of M, where overexpression must not be too deleterious relative to underexpression (see conditions [4] and [5]).

The third, more complicated case occurs when m and M are both present in the population, for example stably maintained at the polymorphic equilibrium given by (3). In this case, we were unable to derive analytic conditions for the spread of I. However, the relative fitnesses of the various genotypes lead to the following four contentions concerning this scenario. First, the average fitness of Ii and II females will increase monotonically with increasing frequency of M. Beyond a certain threshold frequency of M, I may be positively selected. The condition for I to spread will be more restrictive than condition (11). This is because I will also occur in females bearing one or two m alleles, on which background I is more deleterious than in combination with MM. Second, spread of I will ameliorate the negative effects of M in females and thereby strengthen selection for M. Thus, by a process of mutual reinforcement, the frequency of both I and M will increase. Third, because I is neutral in males and not harmful in II females, and because I and M interact in a positive manner with regards to fitness, I will always spread to fixation once it invades the population. Finally, once I has spread to fixation, sexual antagonism with regard to M disappears and both M/Y males and MM females have the highest fitness within each sex. Thus, M will spread to fixation along with I. In summary, I may invade the population if M is at a sufficiently high initial frequency, and if this happens, both M and I will sweep to fixation.

This assertion is supported by numerical iterations of the recursion equations. An example for the dynamics when I is introduced into a population that has reached an internal equilibrium at the **M** locus is shown in Figure 2. It can be seen how the frequency of the M allele approaches the polymorphic equilibrium given in equation (2). Introduction of I then leads to fixation of both I and M. We expect that this reinforcement generalizes for any polymorphic equilibrium of two alleles M_1 and M_2, that is, invasion of I should generally lead to increased frequency of the M allele with the higher value of αc.

The impact of the recombination rate r on the spread of I in cases with polymorphism at the M locus depends on whether I arises on a chromosome bearing m or M, that is, on the initial linkage disequilibrium. Simulations indicate that if I is initially associated with m, recombination rate is important mainly for the speed of invasion of I, but not for the condition of invasion: the lower r, the slower invasion of I because it takes longer until I becomes sufficiently associated with M so that it is selected for. Only if there is complete linkage between the two loci ($r = 0$), spread of I is prevented. On the other hand, if I arises on a M bearing chromosome, linkage will favor the spread of I. More precisely, our simulations indicate that in this case, there may be a threshold recombination rate below which I can spread, but above which spread is not possible (results not shown). This can be understood from the mutual reinforcement of M and I. Although I may not be favored when in linkage equilibrium because of its adverse effects in combination with m, prolonged association with M drives up the frequency of both M and I, thus ameliorating the negative effects of I.

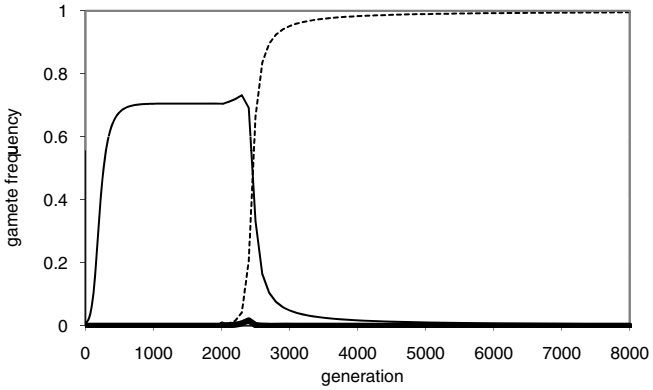

Figure 2. Example for invasion of the upregulating allele *M*, spread to polymorphic equilibrium, and subsequent spread of X-inactivating allele *I*. Shown are the dynamics for gamete frequencies in females, x_{Mi} (solid line), x_{mI} (bold line), and x_{MI} (dotted line). The population was initiated with gamete frequencies (x_{mi}, x_{Mi}, x_{mI}, x_{MI}, y_{mi}, y_{Mi}, y_{mI}, y_{MI}) = (0.99, 0.01, 0, 0, 1, 0, 0, 0). At generation 2000, the inactivating allele *I* was introduced at frequency 0.01 in females. Parameters take the values $w(1) = 0.9$, $w(1 + \alpha c/2) = 0.97$, $w(1 + \alpha c) = 0.989$, $w(1 + c) = 0.99$, $w(2 + \alpha c) = 0.99$, $w(2 + 2\alpha c) = 0.93$, and $r = 0.1$.

Finally, we can ask what the evolutionarily stable state of the system is provided the I allele has become fixed. From equation (7), the condition for an allele M_1 to be stable against invasion of an allele M_2 when I is fixed is

$$\frac{w(1+c_2)-w(1+c_1)}{w(1+c_1)} \leq 2\left\{\frac{w(1+\alpha_1 c_1)-w(1+(\alpha_1 c_1+\alpha_2 c_2)/2)}{w(1+(\alpha_1 c_1+\alpha_2 c_2)/2)}\right\}. \quad (12)$$

Because for any $c_2 > c_1$ and $\alpha_2 > \alpha_1$ the left side of this inequality is positive and the right side is negative, the evolutionarily stable allele $\tilde{M}$ is characterized by $\tilde{c} = \tilde{\alpha} = 1$. This is readily understood intuitively because with XCI, dosage is optimized in males and females without any constraints imposed by sexual conflict.

Discussion

We have constructed a simple mathematical version of Charlesworth's (1978, 1996) verbal model of the evolution of XCI. We did not model the initial degeneration of genes on the Y chromosome but assume that this has occurred and that, as a result, males (with a single active allele) have suboptimal levels of expression whereas females (with two active alleles) have the optimal level of expression. In terms of our model, the first step is the spread of an allele that upregulates expression of an X-linked locus in males, but also (to some extent) in females.

This upregulating allele will spread only if the fitness reduction in females is small relative to the fitness increase in males. In a second step, another allele (at a different X-linked locus) spreads that inactivates the focal locus on the X chromosome in females. The selective forces required for this step to occur are opposite to those involved in the first step: overexpression in females must be deleterious, whereas underexpression (which ensues in females following XCI if the preceding upregulation of gene expression is less than twofold) must not be too deleterious. As a result, a certain balance between the fitness values of the various genotypes is required for the entire process to work. (Note that although strong selection for non–sex-specific upregulation tends to imply weak selection for inactivation once the upregulating allele *M* has become fixed, the presence of the inactivating allele in the population reinforces selection on *M*, and vice versa.)

Analysis of the evolutionary dynamics of our model, in which we considered the recurrent emergence of new mutations at the **M** locus, demonstrated the existence of two evolutionarily stable states. Both states achieve optimal expression in both sexes, although by different mechanisms. The first equilibrium is represented by fixation of an allele with parameters $c = 1$ and $\alpha = 0$ at the **M** locus, and the (noninactivating) *i* allele at the inactivating locus. With this genotype, upregulation is twofold and occurs in males only. Roughly speaking, this state resembles the mechanism of dosage compensation observed in *D. melanogaster* (Baker et al. 1994). The second equilibrium is reached when an *M* allele with $c = 1$ and $\alpha = 1$ at the focal locus and the *I* allele at the inactivating locus are fixed. Here, upregulation is again twofold but completely independent of sex, and one allele at the focal locus is inactivated in females. The probability that the second of these states—corresponding to the mammalian system of XCI—is reached will depend on both the shape of the fitness function and the rates at which the various possible mutations are produced at the two loci. In particular, for XCI to evolve, evolution of sex-specific gene expression (*M* alleles with low values of α) must be slow, and overexpression needs to be only mildly deleterious.

We have made two assumptions about the mechanism of XCI. First, in females heterozygous for the inactivating allele, we assumed that the X chromosome (i.e., the focal locus on that chromosome) where the inactivating allele sits is the one that is inactivated. Several other patterns are logically possible, for example, inactivation of the opposite, the paternal, or a randomly chosen X. However, we do not expect the choice of the X chromosome to be inactivated to influence our results. This is because in most cases, we expect fixation of the upregulating allele to occur before invasion of the inactivating allele, so that both X chromosomes are always equivalent with respect to the focal locus. Moreover, even when the inactivating allele invades a population polymorphic for the upregulating allele, our results should not be affected qualitatively as long as there is recombination between the two loci.

Second, and more importantly, we assume that only one X chromosome is inactivated in females homozygous for the inactivating allele. Without this assumption, homozygous females would have dosage $z = 0$ at the focal locus. Because this is expected to be deleterious, there would be overdominance with regards to selection for the inactivating allele. As a result, the inactivating allele may spread when rare, but will be selected against at higher frequencies and thus reach a polymorphic equilibrium. Two distinct mechanisms exist to ensure that one X chromosome remains active in somatic cells of female mammals. Eutherians employ a counting and choice mechanism that ensures a randomly selected X remains active (Heard and Disteche 2006). The existence of such a sophisticated mechanism in the initial stages of the evolution of XCI seems implausible. Marsupials, however, employ a simpler mechanism in which paternally derived X chromosomes are inactivated (Graves 1996). Such a mechanism does not require "counting" and seems more plausible for early stages in the evolution of XCI. It might, for example, result from a carryover of X inactivation during spermatogenesis into the next generation (Lifschytz and Lindsley 1972). We do not, however, expect inactivation of the paternally derived X chromosome (rather than a randomly chosen as in our model) in females homozygous for the inactivating allele to qualitatively influence our predictions.

Our model has considered only a single locus under dosage-dependent selection. By contrast, XCI is known to cover a large fraction of the mammalian X chromosome, affecting hundreds of genes simultaneously. Therefore, it is important to consider the extent to which our results are applicable to a multilocus case. It is clear that gene expression can be regulated locally by the gene itself (its promoter) or by elements nearby, making plausible our assumption that gene expression is regulated in *cis*. Moreover, several X-linked genes are known that escape inactivation, whereas adjacent genes are inactivated (Carrel and Willard 2005). Thus, whether a particular region of the X chromosome is inactivated or not appears at least partly to be determined locally. Nevertheless, it is possible that the initial inactivating mutation affected alleles at multiple loci, each with idiosyncratic values of *c* and α. The success of the inactivating mutation would then be determined by the effects on female fitness of changes in expression of multiple proteins, some changes are beneficial and others deleterious, and of epistatic interactions among these gene products. Further theoretical analyses are required to determine whether our simple model provides a reasonable guide to this more complex situation.

In humans, as many as 15% of X-linked genes consistently escape inactivation; another 10% are inactivated in some females, but not in others (Carrel and Willard 2005). However, this is to a large extent explained by the evolutionary history of the sex

chromosome. Several evolutionary strata have been identified on the mammalian X chromosome according to the timing when suppression of recombination between the sex chromosomes occurred and the two sex chromosomes commenced to evolve independently (Lahn and Page 1999; Sandstedt and Tucker 2004). Although in the oldest stratum of the human X chromosome, almost all genes are inactivated, the majority of genes in the most recent stratum escape inactivation (Carrel and Willard 2005). In mice, only seven genes are known to escape inactivation, and five of these have functional homologues on the Y chromosome (Chow et al. 2005).

Thus, it appears that over evolutionary time, almost all genes on the mammalian X without functional homologues on the Y chromosome are eventually inactivated. However, in the light of our results and the above considerations about the evolutionary dynamics of XCI, there appears to be a problem with the view of gene-by-gene inactivation. Why do not some genes reach a state of male-specific upregulation as in *D. melanogaster*, and others a state of X-inactivation? There are at least two possible answers to this question, which are not mutually exclusive. First, restricting upregulation of gene expression of an X-linked locus to males might be much harder to evolve than extending a pre-existing mechanism of XCI to that particular locus in females. However, given that gene expression in mammals seems to be sex-biased at many loci (Yang et al. 2006), this explanation is unappealing. Second, the fitness functions for mammalian X-linked genes (depending on dosage) might be particularly conducive to equal upregulation in both sexes, because overexpression of genes in females may lead to little or no reduction of fitness. Again, we do not think this to be a likely explanation, as we expect a large variety of fitness functions for different genes both with respect to general steepness and symmetry around the optimal level of gene expression. In addition, if overexpression leads to only minor fitness reductions, selection for XCI will be weak.

Haig (2006) recently proposed a parental antagonism model (PAM) as an alternative explanation to SAM for the evolution of XCI. PAM is related to the kinship theory of genomic imprinting (reviewed in Haig 2000, 2004) and has at its core the conflict between maternally and paternally derived genes over resource allocation from the mother. The main premise of PAM is that X chromosomes tend to accumulate embryonic growth inhibitors. This leads to selection for inactivation of X chromosomes when paternally derived. In response to this process, expression of maternally derived growth inhibitors will increase so that expression levels optimal for maternally derived genes will be restored. Finally, a transition from paternal to random X-inactivation (which does not affect overall expression levels) may be favored because of the disadvantages of functional haploidy.

The main appeal of PAM is that it provides an explanation for why XCI is specific to mammals, including the occurrence of imprinted XCI in marsupials. Furthermore, PAM accounts for mechanistic links between XCI and genomic imprinting (Lyon 1999; Lee 2003; Reik and Lewis 2005). The results presented in this article show that SAM does work in principle, although it requires particular quantitative relations between the costs of underexpression in males and overexpression in females. PAM has not yet been subject to even this limited degree of formal modelling. A choice between the models must await future theoretical analyses and empirical evidence about the kinds of genes expressed on X chromosomes (in particular whether the X chromosome is enriched for inhibitors of offspring demand).

We should emphasize that the SAM for the evolution of XCI has not been formally modelled in the 30 years since it was first proposed. Our model, in this article, is the first formal attempt to understand this evolutionary process. It is not intended to be a definitive exposition of SAM but is intended to stimulate further empirical and theoretical investigations.

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