Evolutionary Genetic Models of the Ovarian Time Bomb Hypothesis for the Evolution of Genomic Imprinting

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ABSTRACT

At a small number of loci in eutherian mammals, only one of the two copies of a gene is expressed; the other is silenced. Such loci are said to be “imprinted,” with some having the maternally inherited allele inactivated and others showing paternal inactivation. Several hypotheses have been proposed to explain how such a genetic system could evolve in the face of the selective advantages of diploidy. In this study, we examine the “ovarian time bomb” hypothesis, which proposes that imprinting arose through selection for reduced risk of ovarian trophoblastic disease in females. We present three evolutionary genetic models that incorporate both this selection pressure and the effect of deleterious mutations to elucidate the conditions under which imprinting could evolve. Our findings suggest that the ovarian time bomb hypothesis can explain why some growth-enhancing genes active in early embryogenesis [e.g., mouse insulin-like growth factor 2 (Igf2)] have evolved to be maternally rather than paternally inactive and why the opposite imprinting status has evolved at some growth-inhibiting loci [e.g., mouse insulin-like growth factor 2 receptor (Igf2r)].

The unequal expression in mammals of some maternally and paternally derived genes known as genomic imprinting reduces (or even eliminates) the masking benefits of diploidy. (For a review of the benefits of diploidy over haploidy see Perrot et al. 1991; Otto and Goldstein 1992.) Consequently, genomic imprinting confers an apparent selective disadvantage on any imprinted individual and yet several mammalian loci appear to have evolved from a nonimprinting state to become imprinted (Bartolomei and Tilghman 1997). Several hypotheses have been proposed to explain this paradox (reviewed in Haig and Trivers 1995; Hurst 1997; see also Spencer et al. 1999; Spencer 2000). One of the earliest suggestions notes that there are no parthenogenetic mammals and that even attempts to create such lines in the laboratory have not succeeded (Kaufman 1983). The apparent restriction of imprinting among vertebrates to mammals, therefore, has led to the hypothesis that imprinting, by requiring genetic input from both parents, evolved to prevent parthenogenesis (Solter 1988).

A number of criticisms of this hypothesis can be made (Solter 1988; Haig and Trivers 1995; Hurst 1997). Clearly, only maternal inactivation of an allele could have the necessary feature of killing a parthenogenetic embryo. But by destroying some fraction of its carriers’ progeny, such an allele would actually decrease its frequency relative to a nonimprinting allele that permitted asexual reproduction (Haig and Trivers 1995; Hurst 1997). Thus, selection at the level of the individual would oppose the evolution of imprinting to prevent parthenogenesis. By suggesting that parthenogenetic lines are evolutionary dead ends that should be selectively eliminated, the parthenogenesis-prevention hypothesis apparently requires group-level selection, which would be easily subverted by individual-level selection. As a result, the parthenogenesis-prevention hypothesis has generally been considered insufficient to account for observed patterns of imprinting (Haig and Trivers 1995; Hurst 1997).

Nevertheless, one version of this suggestion, the ovarian time bomb hypothesis (OTBH; Varmuza and Mann 1994), explicitly envisages an individual-level cost of parthenogenesis, namely ovarian trophoblastic disease. This cancer-like condition could arise as a consequence of an unfertilized egg spontaneously developing in the oocytes. Inactivating or downregulating the maternal copy of a growth-enhancing gene in a mother’s offspring means that a paternal genetic contribution is essential for successful embryogenesis. Hence, this form of imprinting in a mother can prevent such rogue reproduction and confers on her a selective advantage over those genotypes that are not imprinted.

In this article, we develop three evolutionary genetic models of how imprinting could evolve under the assumptions of the OTBH. These models allow us to predict when an imprintable allele can invade a population originally fixed for a nonimprintable ancestral allele, when the nonimprinting allele can invade a population
fixed for imprinting, and when the two alleles can coexist. The first model describes the case of maternal inactivation; the others deal with paternal inactivation.

MODEL FORMULATION AND ANALYSIS

Model 1: Maternal inactivation and deleterious mutation:
This model adapts the analysis of Spencer et al. (1998) of the genetic conflict hypothesis to the ovarian time bomb hypothesis. Consider an autosomal growth-factor locus originally fixed for an imprintable allele A. Now suppose a maternally imprintable allele a is introduced. Under the OTBH, this new allele would enjoy a selective advantage because, when present in unfertilized eggs, it prevents those eggs from spontaneously developing and thereby reduces that female’s risk of ovarian trophoblast disease. We can therefore write the respective fitnesses of AA and aa females as 1 − s and 1, respectively (s ≤ 1). Heterozygote females would enjoy an intermediate fitness of 1 − hs; in the absence of segregation distortion, the imprintable allele should be passed to one-half of a heterozygote female’s eggs, so we would expect h = ½. Males, of course, experience no cancer risk from expressing the growth factor in their gametes, so all of their fitnesses would be unity.

Under this simple model, the imprintable allele a always confers a selective advantage over the non-imprintable allele and should therefore become fixed in the population. This analysis, however, fails to consider the costs of haploidy, particularly the loss of masking of recessive deleterious mutants (Perrot et al. 1991; Otto and Goldstein 1992). Consider a recessive mutant allele a* that arises from both A and a at rate μ. This mutation will be completely masked in both Aa* and a*A individuals, where we write the paternally derived allele first. It will also be masked in aa* individuals. However, a*a(a) individuals, where the parentheses denote inactivation of the maternally derived allele, will suffer a reduced fitness of 1 − t (t ≤ 1) because they lack a functional copy of the gene. Note that this selection pressure, unlike that imposed by ovarian cancer, applies equally to both sexes. Assuming that a* acts like a null allele, this mutant will also confer a reduced risk of cancer, since it fails to produce sufficient growth factor to initiate development when present in an unfertilized egg. We can then construct Table 1 to list the nine distinct genotypes and their relative fitnesses in both females and males.

Let us define f1, f2, and f3 as the respective frequencies of the A, a, and a* alleles in females and m1, m2, and m3 as the corresponding frequencies in males (thus, Σf = Σm = 1). We then derive the following recursions for allele frequencies in the following generation,

\[ T_f^1 = \frac{1}{2}(1 - \mu)\{(1 - h)s(f_1 + m_1 - 2sf_1m_1(1 - h))\} \quad (1a) \]

\[ T_m^1 = \frac{1}{2}(1 - \mu)(f_1 + m_1) \quad (1b) \]

The paternal allele is written first, and parentheses denote an imprintable allele that has been (maternally) inactivated.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency</th>
<th>Female fitness (Wf)</th>
<th>Male fitness (Wm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>m₁f₁</td>
<td>1 − s</td>
<td>1</td>
</tr>
<tr>
<td>A(a)</td>
<td>m₁f₂</td>
<td>1 − hs</td>
<td>1</td>
</tr>
<tr>
<td>Aa*</td>
<td>m₁f₃</td>
<td>1 − hs</td>
<td>1</td>
</tr>
<tr>
<td>aA</td>
<td>m₂f₁</td>
<td>1 − hs</td>
<td>1</td>
</tr>
<tr>
<td>a(a)</td>
<td>m₂f₂</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>aa*</td>
<td>m₂f₃</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>a*AA</td>
<td>m₃f₁</td>
<td>1 − hs</td>
<td>1</td>
</tr>
<tr>
<td>a*aa</td>
<td>m₃f₂</td>
<td>1 − t</td>
<td>1 − t</td>
</tr>
<tr>
<td>a<em>aa</em></td>
<td>m₃f₃</td>
<td>1 − t</td>
<td>1 − t</td>
</tr>
</tbody>
</table>

The paternal allele is written first, and parentheses denote an imprintable allele that has been (maternally) inactivated.

This system of equations has multiple equilibria (i.e., values of f₁, f₂, f₃, m₁, m₂, and m₃ that satisfy Equations 1 with the primes removed from the left-hand sides). However, several of these equilibria are associated with allele frequencies that are complex, less than zero, or greater than unity. The only four biologically feasible solutions are: (i) the fixation of a* (i.e., f₁ = f₂ = m₁ = m₂ = 0, f₃ = m₃ = 1), which we denote equilibrium M1; (ii) a mutation-selection balance between a and a*, denoted M2 and given by f₂ = m₂ = 1 − 2μ/γ(1 + μ) and f₃ = m₃ = 2μ/γ(1 + μ); (iii) a mutation-selection balance between A and a*, denoted M3 (see Appendix A for allele frequencies); and (iv) a mutation-selection balance among all three alleles, denoted M4 (see Appendix A for allele frequencies). Figure 1 plots these equilibria.
and the system’s evolutionary trajectories for a representative set of parameter values.

Stability analysis: Near equilibrium M1 (fixation of $a^*$), we linearize the system (1) and solve for the eigenvalue that governs increase of $A$ when it is rare. This eigenvalue is less than unity, and equilibrium M1 is stable to invasion by $A$, if

$$t < \mu + \frac{1}{2}hs(1-\mu). \quad (2a)$$

Using the same procedure, we find that equilibrium M1 is stable to invasion by $a$ if

$$t < \frac{2\mu}{1 + \mu}. \quad (2b)$$

At equilibrium M2 ($a/a^*$ polymorphism, $A$ absent), the reduced two-allele system is stable when inequality (2b) is reversed; this condition is the same as that under which the allele frequencies at equilibrium M2 are biologically feasible (i.e., $0 \leq f_2, m_2, f_3, m_3 \leq 1$), so the reduced ($a/a^*$) system is always stable. This same equilibrium is stable to invasion by $A$ if

$$hs > \frac{2\mu}{1 + \mu}. \quad (2c)$$

For the two remaining equilibria, we explicitly assume $h = \frac{1}{2}$ for the sake of algebraic tractability. Numerical iteration of recursions (1) indicates that equilibrium M3 ($A/a^*$ polymorphism, $a$ absent) exists only when equilibrium M1 is unstable to invasion by $A$ [i.e., $t > \frac{s}{2} + \mu(4-s)/4$, the reverse of (2a)]. In fact, M3 is the root of the fourth-degree polynomial given by (A2) in Appendix A. It is a matter of algebra to see that to order $\mu$, this polynomial is positive at $f_1 = 1$ and, if $t > \frac{s}{2} + \mu(4-s)/4$, it is negative at $f_1 = 0$. Hence, there is at least one mutation-selection balance equilibrium $M3$. For reasonable $\mu$ values, we have found no other solutions for an $A/a^*$ mutation-selection equilibrium. Further numerical investigation suggests that M3 is stable to invasion by allele $a$ when

$$t > \frac{1}{2}[s - \mu(4-s)] \quad (2d)$$

and

$$t > \frac{s[(16 - 56\mu - 4s(4 - 13\mu) + 3s^2] - 4s^3}{80(1 + 5\mu) + 80s^2 - 35s^3}. \quad (2e)$$

Note that all terms in the denominator of (2e) include a factor $\mu$, but that some terms in the numerator do not. For typical values of $\mu$, therefore, this inequality greatly restricts the values of $s$ and/or $t$ at which M3 is stable to $a$ (e.g., if $\mu = 10^{-6}$, then the requirement that $t \geq 1$ implies $s < 2.9 \times 10^{-3}$).

Inequality (2e) is also relevant for the existence of M4, the internal equilibrium, which is given by a cubic equation that factors to give the value of $f_1$ reported in (A33) of Appendix A. After some algebra, the allele frequencies at this equilibrium are seen to be biologically feasible if both (2c) with $h = \frac{1}{2}$ and (2e) hold. We have not been able to derive analytical conditions for the local stability of M4. However, the fact that its existence entails the local stability of M2 and M3 suggests that it should be unstable when it exists. Indeed, there are no values of $s < 1$ for which inequalities (2b), (2c), and (2e) hold simultaneously. For numerical verification of M4’s instability, we set $\mu = 10^{-6}$, allowed both $s$ and $t$ to vary from $10^{-6}$ to 1 in logarithmic increments of $10^{-4}$, and at each such point (set of parameters) determined the equilibrium’s stability. For all points at which each equilibrium M4 was feasible, it was also unstable; for nearly all points at which the equilibrium was unfeasible, it was stable (the exceptions being several cases with $t < 2\mu$). There were no instances when the equilibrium was simultaneously stable and biologically feasible. We also performed simulations for $\sim$50 specific combinations of $s$, $t$, and $\mu$, in which we started the system near equilibrium M4 and then iterated equations (1) over $10^4$ generations. The equilibrium was unstable in all such trials. These combined findings strongly suggest that equilibrium M4 is never stable.

These results are summarized in Figure 2, which plots equilibrium stability over $s$-$t$ phase space (assuming $h = \frac{1}{2}$ and $\mu = 10^{-6}$). From this figure, we see that equilibrium M1 (fixation of $a^*$) is stable only for very small values of $t$, at which selection against mutant alleles is too weak to counteract the pressure of recurrent irreversible mutations. Likewise, only equilibrium M3 ($A/a^*$ polymorphism) is stable for very small values of $s$, at which selection for $A$ alleles to mask mutant $a^*$ alleles overcomes the opposing selection for decreased cancer risk. For
these values of $s$ and $t$, imprinting will neither increase when rare nor be maintained if already present. We contrast this result with the wide range of $s$ and $t$ values at which only equilibrium M2 is stable. In these regions, the decreased cancer risk associated with the $a$ allele more than compensates for the inability to mask $a^b$ alleles, so imprinting should invade and be maintained.

Figure 2 also demonstrates that, for small values of $s$ and slightly larger values of $t$, equilibria M2 and M3 can be simultaneously stable. In this region of phase space, an imprintable allele will be maintained if present but cannot invade. For some sets of selection coefficients, therefore, maternal inactivation and full expression can both be stable evolutionary outcomes. (In a finite population, of course, genetic drift may enable the system to switch from one stable equilibrium to another.) This feature does not appear in verbal statements of the OTBH or in the quantitative-genetic model of Iwasa (1998); it is a novel prediction of our mathematical model. In contrast, the model finds no stable equilibria at which imprinting can be polymorphic (i.e., with both $A$ and $a$ present), contrary to the predictions of a model for the genetic-conflict hypothesis (Spencer et al. 1998).

**Model 2: Paternal inactivation and deleterious mutation:** We can modify the previous model to make the inactivation of the $a$ allele paternal. Verbal arguments based on the OTBH (Varmuza and Mann 1994) imply that imprinting should not evolve in this circumstance. Using Table 2, where the first allele written now represents the maternally derived allele, we can derive the following iterations:

\[
T_i f_i = \frac{1}{2}(1 - \mu) [(1 - s)(f_i + m_i) + s(1 - h)(f_i m_i + f_i m_1)]
\]

(3a)

\[
T_m m_i = \frac{1}{2}(1 - \mu)(f_i + m_i)
\]

(3b)

\[
T_i f_i = \frac{1}{2}(1 - \mu) [(1 - s)(f_i + m_i) + s(1 - h)(f_i m_i + f_i m_2)
+ (s - hs - t + hst)f_i m_3]
\]

(3c)

\[
T_m m_i = \frac{1}{2}(1 - \mu)(f_i + m_i - t f_i m_3)
\]

(3d)

\[
T_i f_i = \frac{1}{2} [(1 - h s)(f_i + m_i - t f_i m_1) + (2 hs - s f_i m_1)]
+ \frac{1}{2} \mu [2 - (1 + h s)(f_i + m_i)
- 2 s(1 - f_i)(1 - m_i)
+ 2 h s f_i m_2 - s(1 - h s)(f_i m_2)]
\]

(3e)

\[
T_m m_i = \frac{1}{2} [(f_i + m_i - t f_i m_2) - 2 f_i m_3 + m_3)]
+ \frac{1}{2} \mu [2 - f_i - m_i - t f_i m_3],
\]

(3f)

where $T_i$ and $T_m$ are the mean viabilities of females and males, respectively, given by

\[
T_i = 1 - s(1 - f_i)(1 - m_i) - hs(f_i + m_i - 2 f_i m_3)
- tf_i (1 - m_i) + hst f_i m_2
\]

(3g)

\[
T_m = 1 - t f_i (1 - m_i)
\]

(3h)

Once again, this system appears to have four biologically feasible equilibria: (i) the fixation of $a^b$ (i.e., $f_i = f_2 = m_i = m_2 = 0$, $f_3 = m_3 = 1$), denoted P1; (ii) a mutation-selection balance between $a$ and $a^b$, denoted P2 (see appendix B for allele frequencies); (iii) a mutation-selection balance between $A$ and $a^b$, denoted P3 (see appendix B for allele frequencies); and (iv) a second mutation-selection balance between $a$ and $a^b$, denoted P4 (see appendix B for allele frequencies).

**Stability analysis:** Using the same procedure as in the maternal inactivation case, we find that equilibrium P1 (fixation of $a^b$) is stable to invasion by $A$ if
This same equilibrium is stable to invasion by $a$ if

$$t < \frac{2\mu + hs(1 - \mu)}{1 + \mu}. \quad (4b)$$

We have been unable to derive explicit stability criteria for the other three equilibria under this model. However, we can elucidate the internal dynamics of the model by means of the following argument. We see from Table 2 that the $A$ and $a$ alleles are selectively neutral when transmitted paternally. Under maternal transmission, however, this neutrality breaks down: A maternal $A$ is favored over an $a$ when paired with a paternal $a^*$ allele. Thus, in the only case where the $A$ and $a$ alleles are not interchangeable, the former has higher fitness.

As a result, $A$ should always tend to replace $a$ whenever both alleles are present, although this process depends on the frequency of the deleterious mutant $a^*$ and may therefore take many generations. Extensive simulations using a wide range of parameter values (data not shown) bear out this conjecture.

In contrast to model 1, numerical iterations of system (3) show that the paternal inactivation model has a fairly simple outcome (Figure 3). We note that equilibrium $P_1$ (fixation of $a^*$) is stable over a much broader range of parameter values than is $M_1$; even for large values of $t$, sufficiently high cancer risk $s$ can lead to the fixation of the deleterious mutant. For most plausible values, however (i.e., where $t$ is much greater than $s$), the only stable equilibrium is $P_3$ ($A/a^*$ polymorphism). For $h = \frac{1}{2}$, equilibrium $P_2$ is always unstable and equilibrium $P_4$ requires biologically unfeasible allele frequencies; consequently, the $a$ allele is always lost. According to this model, therefore, growth-enhancing genes should not evolve paternal inactivation.

We can also use model 2 to analyze the behavior of growth-inhibiting genes. At such a locus, the unimprintable allele $A$ would now enjoy the selective advantage because it reduces both the chance of an egg spontaneously developing and the probability of expressing a deleterious mutant allele. We can therefore describe this system by simply requiring that $s < 0$ (rather than $s > 0$ as at growth-promoting loci). Modifying Tables 1 and 2 appropriately, we see that the $A$ allele now has higher fitness than the $a$ allele under both the maternal and the paternal inactivation models, so the unimprintable allele should always be selectively eliminated. Therefore, our simple model of the OTBH leaves us with no adaptive explanation for observed instances of paternal inactivation (e.g., Bartolomei and Tilghman 1997).

**Model 3: Paternal inactivation, deleterious mutation, and stabilizing selection:** Alternative formulations of the OTBH, more complex than the model described above, have incorporated possible mechanisms for the evolution of paternal inactivation. The “innocent bystander” hypothesis suggests that paternal inactivation results from imprinting machinery aimed at specific physical features of trophoblast-specific genes but also present in some other genes, both paternal and maternal (Varmuza and Mann 1994). It therefore explains paternal imprinting at a molecular but not at an evolutionary level: Until the putative physical targets of the imprinting machinery are identified, the hypothesis remains descriptive rather than predictive. Iwasa (1998) provided an alternative explanation, proposing that the risk of an unflagellated egg spontaneously developing can be reduced by increasing expression of the maternal growth-inhibiting alleles (as well as decreasing expression of her growth-promoting alleles as Varmuza and Mann originally suggested). Stabilizing selection for constant overall levels of growth inhibition in the zygote would then select for the contrary pattern in paternal alleles, i.e., inactivation of inhibitor alleles. To mimic the effect of such stabilizing selection, we begin with the paternal inactivation model of a growth-inhibiting gene, described above. In this analysis, we replace the $a$ allele with a new allele $a'$, which is underexpressed when inherited paternally and overexpressed when inherited maternally. Each $a'$ allele present in a female therefore inhibits spontaneous development in one-half of her unfertilized eggs, increasing her fitness by an amount $r/2$; each unimprintable $A$ allele she carries likewise decreases the risk of such development in one-half of her eggs, decreasing her fitness by an amount $s/2$. (We assume here that $h = \frac{1}{2}$; given that this is a growth-inhibiting locus, we also expect $r > 0 > s$.) Using Table 3, where the first allele written again represents the maternally derived allele, we can derive the following iterations,

$$T_i f_i = \frac{1}{2}(1 - \mu) \left[ \frac{1}{2}(2 - s)(f_1 + m_1) + \frac{1}{2}s(f_1 + m_1) - sf_1 m_1 \right] \quad (5a)$$

**Figure 3.—Existence and stability of equilibria in s-t phase space for paternal inactivation (model 2).** Parameters and notation are as in Figure 2.
TABLE 3  
Genotype frequencies and fitnesses under model 3  
(paternal inactivation, stabilizing selection,  
growth-inhibiting gene)  

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency</th>
<th>Female fitness ((W_f))</th>
<th>Male fitness ((W_m))</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>(f_1m_1)</td>
<td>(1 - s)</td>
<td>(1)</td>
</tr>
<tr>
<td>A(a')</td>
<td>(f_1m_2)</td>
<td>(1 + h(r - s))</td>
<td>(1)</td>
</tr>
<tr>
<td>A(a')</td>
<td>(f_1m_3)</td>
<td>(1 - hs)</td>
<td>(1)</td>
</tr>
<tr>
<td>a'a</td>
<td>(f_2m_1)</td>
<td>(1 + h(r - s))</td>
<td>(1)</td>
</tr>
<tr>
<td>a'a</td>
<td>(f_2m_2)</td>
<td>(1 + r)</td>
<td>(1)</td>
</tr>
<tr>
<td>a'a</td>
<td>(f_2m_3)</td>
<td>(1 + hr)</td>
<td>(1)</td>
</tr>
<tr>
<td>a'(a')</td>
<td>(f_3m_1)</td>
<td>(1 - t)</td>
<td>(1)</td>
</tr>
<tr>
<td>a'(a')</td>
<td>(f_3m_2)</td>
<td>(1 - t)</td>
<td>(1)</td>
</tr>
</tbody>
</table>

The maternal allele is written first, and parentheses denote an imprinted allele that has been (paternally) inactivated.  

\[ T_{a,m} = \frac{1}{2}(1 - \mu)(f_1 + m_1) \]  
\[ T_{a,f} = \frac{1}{2}(1 - \mu)[\frac{1}{2}(2 + r) - s(f_1m_3 + f_2m_1) + 1 - s]\]  
\[ T_{a,m} = \frac{1}{2}(1 - \mu)(f_2 + m_2) \]  
\[ T_{a,f} = \frac{1}{2}[f_3 + 1/2(r(f_1m_3 + f_3m_1)) - \frac{1}{2}s(f_1m_3 + f_3m_1)] \]  
\[ T_{a,m} = \frac{1}{2}(1 - \mu)(f_2 + m_2 - f_3m_1) \]  
\[ T_{a,f} = \frac{1}{2}[f_3 + 1/2(r(f_1m_3 + f_3m_1)) - \frac{1}{2}s(f_1m_3 + f_3m_1)] \]  
\[ T_{a,f} = \frac{1}{2}[f_3 + 1/2(r(f_1m_3 + f_3m_1)) - \frac{1}{2}s(f_1m_3 + f_3m_1)] \]  
\[ T_{a,m} = \frac{1}{2}(1 - \mu)(1 - t) \]  
\[ T_{a,f} = \frac{1}{2}(1 - \mu)(1 - t) \]  

This system has three biologically feasible equilibria: (i) the fixation of \(a'\) (i.e., \(f_1 = f_3 = m_1 = m_2 = 0, f_2 = m_3 = 1\)), denoted S1; (ii) a mutation-selection balance between \(a'\) and \(a'^*\), denoted S2 (see APPENDIX C for allele frequencies); and (iii) a mutation-selection balance between \(A\) and \(a'^*\), denoted S3 (allele frequencies same as P3; see APPENDIX C).  

Stability analysis: Again using the same procedure as in the maternal inactivation case, we find that equilibrium S1 (fixation of \(a'^*\)) is stable to invasion by A if  
\[ t < \frac{s + 4\mu - \mu s}{2 + 2\mu}. \]

Turning to equilibrium S2 (\(a'/a'^*\) polymorphism), we find that this equilibrium is feasible precisely when inequality (6b) is violated. Within the reduced system involving only the \(a'\) and \(a'^*\) alleles, S1 and S2 are the only two equilibria, so S2 must be stable when S1 is unstable and vice versa. We tested this result by setting \(\mu = 10^{-6}\), allowing \(r\) to vary from \(10^{-7}\) to 1 in logarithmic increments of \(10^{1/4}\); at each such point, we calculated the critical value of \(t\) given by (6b) and also determined via simulation the value of \(r\) at which S2 became unstable. In all cases, the two values were equal, confirming that S2 is internally stable whenever it is feasible. For equilibrium S3, an analogous set of simulations (varying \(s\) from \(-1\) to \(-10^{-7}\) demonstrated that this equilibrium is both feasible and internally stable precisely when inequality (6a) is violated, i.e., when S1 is unstable to invasion by A.  

Now, when both S2 and S3 are feasible, one must be stable and the other unstable (since S1 is unstable and no other equilibria exist). We performed 40 simulations using random parameter values \(-1 < s < 0 < r, t < 1\); in each case, S2 was stable when \(r + s > 0\) and unstable when \(r + s < 0\). We tested this pattern by performing 20 additional simulations with \(r = -s + 0.01\) and 20 with \(r = -s - 0.01\); the former group of simulations all converged to equilibrium S2 and the latter to S3. We conclude that S2 is stable and S3 unstable for \(r + s > 0\) and that the reverse holds true for \(r + s < 0\). (When \(r + s = 0\), the “overexpression” of maternally inherited \(a'\) alleles is equal to the normal expression of \(A\) alleles; this case is therefore equivalent to paternal inactivation without stabilizing selection, which we have already examined under model 2.)  

It is instructive to compare the feasibility of equilibria in models 2 and 3 (Figures 3 and 4, respectively). Because model 3 includes a parameter \(r\) not present in model 2, the former plot has one more dimension than the latter. Moreover, this extra parameter effectively decouples the system’s equilibria: The feasibility of S2 depends on \(r\) but not on \(s\), and the feasibility of S3 depends on \(s\) but not on \(r\), whereas the feasibilities of P2 and P3 from model 2 both depend on \(s\). As a result of this decoupling, equilibrium S2 (mutation-selection balance of the imprinted allele under stabilizing selection) is stable over a wide range of parameter space, although P2 (the corresponding equilibrium without stabilizing selection), as we observed, is never stable. This analysis therefore elucidates the range of parameter values for which IWASA’S (1998) modified version of the OTBH holds.
**DISCUSSION**

The results of our models validate the most basic prediction of the ovarian time bomb hypothesis—that the risk of ovarian cancer in females can lead to the evolution of maternal inactivation. The feasibility of such an evolutionary event will obviously depend on the parameter values present in a particular system. Specifically, imprinting of growth-enhancing genes can evolve most easily when the cancer risk, \( s \), exceeds a specific threshold, calculated from (2e). Once imprinting has evolved, however, it can be maintained even if \( s \) decreases significantly below that threshold. Moreover, our results imply that polymorphism in imprinting status will not evolve under the selection regime envisaged by the OTBH, a finding in contrast to that of a similar model for the genetic-conflict hypothesis (Spencer et al. 1998). Our models also confirm the verbal hypothesis in predicting that the original form of the OTBH should not lead to paternal imprinting of growth-enhancing genes.

The models also suggest possible answers to some objections that have been raised against the OTBH. One such objection states that mammalian ovarian teratomas are too rare to apply the selective pressure needed to fix an imprinted allele (Solter 1994). Our first model implies that if this pressure falls below a threshold (\( s = 4\mu/(1 + \mu) \)), selection should indeed be too weak to maintain imprinting. For realistic mutation rates, however, this threshold is very low (e.g., if \( \mu = 10^{-6} \), then \( s \approx 4 \times 10^{-10} \)). We therefore conclude that the OTBH can explain the maintenance of imprinting even when the risk of ovarian cancer is very low, although that risk must be higher for imprinting to evolve in the first place.

A second objection that has been raised to the OTBH suggests that a single maternally imprinted growth-factor locus would avert the risk of ovarian trophoblast disease and that the existence of multiple such alleles represents evidence against the hypothesis (Haig 1994; Haig and Trivers 1995). This argument is valid if imprinting at one locus can effectively eliminate all possibility of unfertilized eggs spontaneously developing; however, it seems feasible that the effect could be less definitive (Iwasa 1998). We can generalize our model by assuming the existence of \( N \) loci with imprintable alleles, each of which reduces the fitness of a homozygous unimprinted female by amount \( s \). As each imprintable allele becomes fixed, the selective pressure imposed by the remaining cancer risk is reduced, until the risk becomes too low for further imprinting alleles to invade. Given the ease with which selection at a single locus can exceed the invasion threshold given by inequality (2e), our first model suggests that imprinting could easily evolve at multiple loci via the OTBH mechanism. Thus, the OTBH can be consistent with the presence of multiple imprinting alleles (see also Iwasa 1998).

A third objection notes that while the original version of the ovarian time bomb hypothesis might elucidate a mechanism for maternal imprinting, it cannot explain the inactivation of paternal alleles (Haig 1994; Hurst 1997), except by positing that such alleles might be “innocent bystanders” (Varmuza and Mann 1994). Indeed, our second model demonstrates that neither growth-enhancing nor growth-inhibiting genes should evolve paternal inactivation under the original form of the OTBH. Our third model, however, confirmed the
validity of Iwasa’s (1998) suggestion that paternal inactivation of growth-inhibiting genes could evolve from stabilizing selection augmenting the standard OTBH. This revised version of the OTBH is therefore a viable explanation for the evolution of both maternal and paternal inactivation of growth-affecting loci in mammals.

The OTBH has also been criticized for its inability to explain imprinting in organisms other than mammals, for example, in insects and plants (Haig 1994; Moore 1994). Clearly, the reproductive systems of these groups rule out the possibility of ovarian cancer and hence any selective advantage of imprinting under the assumptions of this hypothesis. Mann and Varmuza (1994) suggest that imprinting in different phyla may have arisen for different reasons. We can only concur that, regardless of the OTBH’s ability to explain the evolution of mammalian imprinting, alternative hypotheses may be needed to account for the same phenomenon in other taxonomic groups.

Using evolutionary-genetic models to evaluate verbal hypotheses for the evolution of imprinting has been controversial. Haig (1999), for instance, claimed that such models are inappropriate because they do not apply to long-term evolutionary change. In his view, only game-theoretic models should be used to investigate hypotheses for the evolutionary origin of imprinting. There are two difficulties with Haig’s (1999) analysis, however, the first about the relationship between the two classes of models and the second concerning his description of the sort of evolutionary genetic model used above. This article is not the place for a full comparison of the applicability domains of game-theoretic and evolutionary-genetic models, but it is sufficient here to point out that the relationship between them is more complex than Haig (1999) implies and that game-theoretic models can potentially be misleading. In the context of imprinting, the models of Spencer et al. (1998; see also Spencer 2000) constructed genotypic fitnesses and tracked the dynamics of genotype frequencies. It is well known (see, e.g., Cavalli-Sforza and Feldman 1978) that these dynamics will often differ from those believed to follow from the use of inclusive-fitness arguments such as those of Haig (1992, 1997). The latter described a game “in which the players are alleles at a locus, strategies are alleles’ patterns of expression . . .” (Haig 1999, p. 1229). As these arguments reduce to initial increase properties of alleles, they cannot be expected to reveal the more complex dynamics of differential genotypic fitnesses. The same restrictions apply to the analyses of Mochizuki et al. (1996) and Iwasa and Pomiankowski (2001), whose models of quantitative genetic determination of imprinting also ignore the dynamic complexity that results from full genotypic analysis when fitnesses are determined by genotypes. Thus arguments phrased in terms of an “allele’s strategy” have difficulty coping with evolution that occurs subsequent to the attainment of Spencer et al.’s polymorphic equilibrium. This problem is common to a number of genetic-conflict models where the treatment involves more complex genetics, such as two linked loci (Eshel and Feldman 1984; Eshel et al. 1998).

The second of Haig’s (1999) criticisms concerned Spencer et al.’s (1998) model of the genetic-conflict hypothesis, which considered two alleles, an unimprintable A and an imprintable a. As in the model above, Spencer et al. (1998) were interested in the conditions under which A was displaced by a and vice versa and when the two alleles could coexist. Haig (1999) alleged that this model assumed that a was always completely inactivated when passed on by the imprinting sex and hence only qualitative differences in levels of expression were studied. But none of the algebra used this assumption; indeed Spencer et al. (1998) explicitly pointed out that such a restriction was unnecessary. We point out that a similar generalization applies to the model above: Although we motivate our modeling by describing a as being inactivated when imprinted and a* as a null mutant, the algebra requires neither of these properties to be assumed. (It is crucial, however, that a and a* have similar effects when passed on by the imprinting sex; this assumption could, in principle, be relaxed, at the expense of some sordid algebraic complications.) In the framework of allelic strategies, Haig (1999) is correct in pointing out that by treating the level of imprinting as continuous, he is concerned with long-term evolution in the sense of Eshel and Feldman (1984, 2001; Eshel 1996). However, if the short-term evolutionary scale studied by Spencer et al. (1998) permits polymorphism or prevents allelic fixation (as they showed was indeed possible), then long-term arguments in which evolution steps between such states of fixation may be misleading or even wrong.

These disagreements are important in determining the novelty of our findings. For example, the evolutionary-genetic models developed here and in Spencer et al. (1998) imply that the OTBH and the genetic-conflict hypothesis make an important distinguishing prediction: Polymorphism in imprinting status is present only under the latter. Crucially, this prediction can be made only using evolutionary-genetic models because only they can find polymorphic equilibria. The game-theoretic models are designed to find only fixation equilibria (Haig 1999), whereas Mochizuki et al.’s (1996) and Iwasa’s (1998) quantitative-genetic models follow only the population’s mean levels of gene expression. Our finding that polymorphism is not possible under the OTBH is thus novel because it is tacitly assumed in these other models.

Our models also suggest a second distinguishing prediction between the OTBH and the genetic-conflict hypothesis: The simultaneous stability of imprinting and nonimprinting for biologically plausible parameter values occurs only under the OTBH. The possibility of such a bistable system has been previously noted in
a quantitative-genetic study of the genetic-conflict hypothesis (Mochizuki et al. 1996), although those authors note that this occurs only for a narrow range of parameter values (which are of questionable biological relevance; Spencer 2000). Under the present model, the simultaneous equilibria exist over a large and biologically plausible region of parameter space; moreover, the model requires no assumptions about multiple paternity or stabilizing selection. In principle, such a bistable system could explain interspecific variation in imprinting patterns, although we agree with Iwasa (1998) that parameter-value differences between species (e.g., at human and mouse Igf2r) seem a more plausible explanation.

Hence, given the polymorphic imprinting status of both the IGF2R gene (Xu et al. 1993) and the WT1 gene (Jinno et al. 1994) in humans, our models appear to lend greater support to the genetic-conflict hypothesis than to the ovarian time bomb hypothesis, at least for these loci.

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APPENDIX A: MATERNAL INACTIVATION

To derive equilibrium allele frequencies under model 1 (maternal inactivation), we plugged (1g) and (1h) into (1a–1d), substituted $f_1 = 1 - f_f - f_f$ and $m_1 = 1 - m_f - m_f$, and set $p_1 = f_f$, $m_1 = m_f$, $f_f = f_f$ and $m_1 = m_f$ to obtain

$$2f_1[1 - sf_1m_1 - hs(f_f + m_f - 2f_1m_1) - t(1 - f_f)(1 - m_f - m_f)] = (1 - \mu)(1 - h[s_1] + 2[1 - h]sf_1m_1], \quad (A1)$$

$$2m_1[1 - t(1 - f_f)(1 - m_f - m_f)] = (1 - \mu)(s_f + m_f), \quad (A2)$$

$$2f_1[1 - sf_1m_1 - hs(f_f + m_f - 2f_1m_1) - t(1 - f_f)(1 - m_f - m_f)] = (1 - \mu)[f_f + m_f - hsf_1f_1 + f_1m_1 - t(1 - m_1 - m_f)], \quad (A3)$$

$$2m_1[1 - t(1 - f_f)(1 - m_f - m_f)] = (1 - \mu)[f_f + m_f - t(1 - m_1 - m_f)]. \quad (A4)$$

Solving (A2) for $m_f$, we find

Case IA: $f_f = 1, m_f = 1 - \frac{\mu}{1 + \mu}$ \quad (A5)

Case IB: $f_f = m_f = 0$ \quad (A6)

Case IC: $m_f = 1 - m_f - \frac{2m_1 - (1 - \mu)[f_f + m_f]}{2t(m_1 - t_f)}. \quad (A7)$

We now examine each of these cases in turn.
Case IA: Plugging (A5) into (A1), we find that
\[
s = \frac{2}{1 + h - \mu(1 - h)} > 1. \quad (A8)
\]
This is an unfeasible value for \( s \); therefore, this case yields no feasible equilibria.

Case IB: Plugging (A6) into (A1) and (A2), we find

Subcase IB1: \( f_z = m_z = 0 \) \hspace{1cm} (A9)
or

Subcase IB2: \( f_z = m_z = 1 - \frac{2\mu}{t} \). \hspace{1cm} (A10)

Subcase IB1 corresponds to equilibrium M1 (fixation which is clearly unfeasible. Similarly, we can write the

Case IC: Plugging (A7) into (A4), we find that

\[
2m_1(1 - \mu) - 2t(1 - f_1)(1 - m_1 - m_z) = 0 \quad (A11)
\]
or

\[
f_z = m_1[1 - \mu - 2t(1 - f_1)(1 - m_1 - m_z)] / \left[ 1 - t(1 - m_1 - m_z) \right]. \quad (A12)
\]

But \( t < 1 \) and \( 0 \leq m_1 - m_z \leq 1 \), so (A11) is clearly unfeasible. We turn instead to (A12), substituting both this equation and (A7) into (A1) to obtain

Subcase IC1:

\[
(1 - \mu)(1 - h) = 2f_1[1 - (1 - \mu)(1 - h)] + 2hf_1 - h - f_1
\]

\[
hs_m(1 - \mu - 2f_1) + f_1(1 - \mu) = 0 \quad (A13)
\]
or

Subcase IC2:

\[
m_z = 2[2f_1[1 - (1 - \mu)(1 - h)] + 2hf_1 - h - f_1] / [2[(1 - \mu)(1 - h) - 2f_1(2h - 1) - f_1 + (1 - \mu)(1 - h)]] . \quad (A14)
\]

(Numerical analysis revealed that the other root of Equation A14 is negative and hence unfeasible.)

Subcase IC1: The first condition presented in (A13) can be written as

\[
(1 - \mu)(2 - s) = -2\mu f_1 \quad (A15)
\]
or

\[
f_1 = \sqrt{4t(1 - \mu - 2h - \mu h)} \pm \sqrt{4\sqrt{t(1 - \mu - 2h - \mu h)}(1 - \mu)(1 - h)}/\{4t(2h - 1)\} . \quad (A16)
\]

Note that the left-hand side of (A15) is positive, while the right-hand side is negative. This solution is therefore unfeasible. We then break (A16) down into two further subcases: \( h < \frac{1}{2} \) (Subcase IC1a) and \( h > \frac{1}{2} \) (Subcase IC1b). [If \( h = \frac{1}{2} \), then (A13) simplifies to (A15), which we have already considered.]

Subcase IC1a (\( h < \frac{1}{2} \)): The discriminant of (A16) can be rewritten as

\[
-4s(1 - 2h) [1 + (1 - s)(1 - 2\mu)] + O(\mu^2). \quad (A17)
\]

For \( h < \frac{1}{2} \), this value will be negative, so the roots of (A16) will be complex and hence unfeasible. Therefore, this subcase has no feasible solutions.

Subcase IC1b (\( h > \frac{1}{2} \)): Define \( Q = 2s(1 - \mu - 2h + \mu h), \ c = 8s(1 - \mu)(1 - hs)(2h - 1), \ d = 4s(2h - 1), \ e = 8\mu^2(1 - h)(2h - 1), \ f = 4\mu^2s^2(1 - h)^2 \). Then \( c, d, e, f > 0 > Q \), so we can write the minus root of (A16)

\[
f_{i-} = -Q - \sqrt{Q^2 + e} / d < -Q - \sqrt{Q^2} / d = 0, \quad (A18)
\]

which is clearly unfeasible. Similarly, we can write the plus root of (A16) as

\[
f_{i+} = \frac{1}{2} + \frac{\mu(1 - h)}{2(2h - 1)} + \sqrt{4s^2(2h - 1)^2} + \frac{e + e + f}{d^2} > \frac{1}{2} + \sqrt{4s^2(2h - 1)^2} = 1, \quad (A19)
\]

which is also unfeasible. Therefore, this subcase also has no feasible solutions, concluding our analysis of Subcase IC1.

Subcase IC2: We can plug (A7) and (A12) into (A3) to obtain

Subcase IC2a: \( m_z = -f_1(1 - \mu) / (1 - \mu - 2f_1) \) \hspace{1cm} (A20)
or

Subcase IC2b:

\[
f_1 - m_1 - \mu(f_1 + m_z) + 2tm_1(1 - f_1)(1 - m_1) = 0 \quad (A21)
\]
or

Subcase IC2c:

\[
2f_1[1 - (1 - \mu)(1 - h) - 2sm_1(1 - 2m_1) - 2sm_1^2]
\]

\[
- f_1[1 - \mu^2 - 4sm_1^2 - hsm_1(3 + 2\mu - \mu^2 - 8m_1 - 4\mu m_1)]
\]

\[
f_1m_1(2\mu(1 - \mu) + m_1(2 - h - 2\mu - hsm_1(4 - \mu)))
\]

\[
m_1^2(1 - \mu)^2 = 0. \quad (A22)
\]

Subcase IC2a: Combining Equations A14 and A20, we find that

\[
(1 - \mu)(1 - hs) = 2[f_1[2hf_1 - h - f_1 + (1 - \mu)(1 - h)]
\]

or

\[
f_1(2 - s + \mu s - 2\mu hs) = (1 - \mu)(2 - s + \mu s(1 - h)). \quad (A24)
\]

Note that (A23) is just the first condition presented in (A13), which has already been analyzed in Subcase IC1. Turning to (A24), we solve for \( f_1 \), then substitute this result into (A20) to solve for \( m_z \), and finally plug these
results into (A7) to solve for $m_2$, obtaining

$$ f_i = \frac{(1 - \mu)(2 - s + \mu s - \mu h s)}{2 - s + \mu s - 2\mu h s}, $$

$$ m_i = \frac{(1 - \mu)(2 - s + \mu s - \mu h s)}{2 - s + \mu s}, $$

$$ m_2 = \frac{2 - 2\mu t - s(1 - \mu)(1 - \mu t + \mu h t)}{t(2 - s + \mu s)}. \quad (A25) $$

By inspection, $m_2 < 0$, so this subcase has no feasible solutions.

**Subcase IC2b:** Substituting (A14) into (A21) and solving for $f_i$, we find that $a f_i^4 + b f_i^2 + c f_i + d f_i + e = 0$, where

$$ a = 2\left(\frac{s}{2}(2h^2 + 4\mu h - 4h^2 - 2ht - 4\mu h + 4h + \mu - 1) \right), $$

$$ b = 2\left(\frac{s}{2}(4\mu^2 h^2 - 6h^2 + 2\mu h t + 10h^2(1 - \mu) \right), $$

$$ c = \left(\frac{s}{2}(2\mu^2 h(1 - h)^2 - 10\mu h^2 + 15\mu^2 h + \mu^3 h^2 t \right), $$

$$ d = \left(\frac{s}{2}(h(1 - \mu)(\mu h - \mu - 4h + 2\mu h - \mu^2 \right), $$

$$ e = \left(\frac{s}{2}(1 - \mu)(1 - t) \right), $$

If we assume $h = \frac{1}{2}$ and discard quadratic and higher-order terms in $\mu$, we obtain

$$ a = -t(2t - 2s + \frac{s^2}{4} - 2\mu t), $$

$$ b = t\left(4t + s - 5s(1 - \mu) - 4\mu t + 3\mu s t - 2\mu s^2 \right), $$

$$ c = \frac{s}{4}(8t + 6s - 12s^2 + 3s^3 - 16\mu t \right), $$

$$ d = \frac{s}{4}[16t(1 - \mu)(1 - t) - 2s(1 + \mu)(1 - 2t) \right), $$

$$ e = -\frac{s}{4}(1 - \mu)(2 - s) (1 - t) \right), $$

Numerical analysis reveals that at most one root of this equation is biologically feasible over the range $0 < s$, $t < 1$, giving a unique solution for $f_i$. Theoretically, we can then calculate other allele frequencies by substituting this value successively into (A14), (A7), and (A20), but this is algebraically cumbersome. An easier approach is to repeat the above analysis under the assumption $f_2 = m_2 = 0$; this procedure gives precisely the same coefficients given in (A26). Both approaches will yield the same solution for $f_i$ and hence for $m_i$, $m_2$, and $f_2$. Since $f_2 = m_2 = 0$ under the second approach, these allele frequencies must be zero under the original derivation as well. Substituting this result into (A7) yields

$$ w_i = \frac{(1 + \mu - 2t(1 - f_i)) + \sqrt{(1 + \mu - 2t(1 - f_i))^2 + 8\mu f_i(1 - f_i)(1 - \mu)}}{4t(1 - f_i)} \quad (A28) $$

(The other root is negative and hence unfeasible.) These allele frequencies define equilibrium M3 (A/* polymorphism). As an alternative derivation, given that $f_2 = m_2 = 0$, we can solve (A2) for $f_i$ and then (A1) for $m_i$, obtaining

$$ f_i = \frac{m_i[1 + \mu - 2t(1 - m_i)]}{1 - \mu - 2tm_i(1 - m_i)} \quad (A29) $$

and

$$ a' m_i^4 + b' m_i^2 + c' m_i + d' m_i + e' = 0, \quad (A30) $$

where

$$ a' = -2t[1 - \mu s - \mu s t \right), $$

$$ b' = 2t(2t - 1 - s(1 + \mu)^2 - 4\mu t \right), $$

$$ c' = -2t(1 - \mu)^2 \right), $$

$$ d' = 4t(1 - \mu)(1 - t) \right), $$

$$ e' = -(1 - \mu)(1 - t)[2t - \mu s \right), $$

This alternative formulation is used to compare model 1 (maternal inactivation) to model 2 (paternal inactivation).

**Subcase IC2c:** Substituting (A14) into (A22) and solving for $f_i$, we find that $a'' f_i^4 + b'' f_i^2 + c'' f_i + d'' = 0$, where

$$ a'' = s(1 - \mu)(1 - 2h)(2 - 4h + h^2 s(1 + \mu)), $$

$$ b'' = s(h^2 s(5 + h^2 s(1 + \mu)) \right), $$

$$ c'' = s(h^2 s(5 + h^2 s(1 + \mu) \right), $$

$$ d'' = h^2(20 + 2s - 16\mu + 3\mu s + 4\mu^2 - 2\mu^2 s - \mu^2), $$

$$ e'' = 2h(9 - 4\mu - 3\mu^2) - 4(1 - \mu^2). $$
\[ e'' = h^2 \left( 1 - 4h + 3\mu - \mu h + \mu^2 - \mu^3 + \mu^3 h \right) + 4\mu(1 - 2\mu)(1 - 2h) + 2s(1 - 6h + 8h^2 + \mu - 3\mu h^2 - \mu^2 + 3\mu^3 h) - 5\mu^2 h^2 - \mu^3 + 3\mu^3 h - 2\mu^3 h^2, \]

\[ d'' = (1 - \mu)(2 - 4h + h^3) \left[ \mu(h(1 + \mu) - 2\mu) \right]. \] (A32)

If we assume \( h = \frac{1}{2} \), then \( d'' = 0 \), so the equation becomes quadratic with the following solutions:

\[ f_i = \frac{1 - \mu}{1 - 3\mu}. \] (A33)

or

\[ f_i = \frac{s + \mu s - 4\mu}{s(1 - 2\mu - \mu^2)}. \] (A34)

Clearly, (A33) yields \( f_i > 1 \), so (A34) is the only feasible root. We can then calculate the other allele frequencies by substituting this value successively into (A14), (A7), and (A12): These frequencies define equilibrium M4 (three-allele polymorphism). We have now examined all subcases, so there are no further equilibria under maternal inactivation.

**APPENDIX B: PATERNAL INACTIVATION**

To derive equilibrium allele frequencies under model 2 (paternal inactivation without stabilizing selection), we plugged (3g) and (3h) into (3a-3d), substituted \( f_2 = 1 - f_1 - f_3 \) and \( m_i = 1 - m_1 - m_2 \), and set \( f_1' = f_1 \), \( m_1' = m_1 \), \( f_2' = f_2 \), and \( m_2' = m_2 \) to obtain

\[
\begin{align*}
2f_1' & = 1 - s(1 - 2h)(f_1 + f_3)(m_1 + m_2) \\
& \quad - hs(f_1 + f_2 + m_1 + m_2) \\
& \quad - t(1 - f_1 - f_3)(1 - m_1 - hsm_2) \\
& = (1 - \mu)[(1 - h)(f_1 + m_1) \\
& \quad - s(1 - h)(2f_1m_1 + f_1m_2 + f_3m_1)], \tag{B1}
\end{align*}
\]

\[
\begin{align*}
2m_2' & = 1 - t(1 - f_1)(1 - m_1 - m_2) \\
& = (1 - \mu)[f_2 + m_2 - tf_2(1 - m_1 - m_2)]. \tag{B4}
\end{align*}
\]

Solving (B2) for \( f_2 \), we find

\[
\begin{align*}
\text{Case IIA: } m_i & = 1, \quad f_2 \left( 1 - \frac{1 - \mu}{1 - \mu} \right) \tag{B5}
\end{align*}
\]

or

\[
\begin{align*}
\text{Case IIB: } f_1 & = m_1 = 0 \quad \tag{B6}
\end{align*}
\]

or

\[
\begin{align*}
\text{Case IIC: } f_2 & = 1 - f_1 - \frac{2m_1 - (1 - \mu)(f_1 + m_1)}{2tm_1(1 - m_1)}. \tag{B7}
\end{align*}
\]

Clearly, (B5) implies that \( f_1 > 1 \); therefore, case IIA yields no feasible equilibria.

**Case IIB:** Plugging (B6) into (B4) and solving for \( m_2 \), we obtain

\[
\begin{align*}
m_2 & = \frac{f_2(1 - \mu)}{(1 + \mu)(1 - t + tf_2)}. \tag{B8}
\end{align*}
\]

Substituting both this value and (B6) into (B3) yields

\[
\begin{align*}
\text{Subcase IIB1: } f_2 & = 0 \tag{B9}
\end{align*}
\]

or

\[
\begin{align*}
\text{Subcase IIB2: } f_2 & = \frac{J \pm \sqrt{R}}{D}. \tag{B10}
\end{align*}
\]

where

\[
\begin{align*}
J & = f^2(3 - 4t + 2t(1 + \mu)) \\
& \quad - s[h(3 - 3t - 2\mu + \mu t + \mu^2) - (1 - \mu)^2], \\
R & = f^2 - 2D(1 - t)(1 + \mu - h(1 - \mu) - 2\mu], \\
D & = 2[s(1 - \mu + 2h(1 - t - \mu)] - t^2(1 + \mu)]. \tag{B11}
\end{align*}
\]

Subcase IIB1 corresponds to equilibrium P1 (fixation of \( a^* \)), while the minus and plus roots of subcase IIB2 correspond to equilibria P2 and P4 (\( a^* / a^* \) mutation-selection balance under paternal inactivation), respectively. Numerical analysis demonstrates that P4 is feasible only if \( h > \frac{1}{2} \); when both P2 and P4 are feasible, \( f_2 \) is greater at the former than at the latter.

**Case IIC:** Solving (B4) for \( m_2 \), we find that

\[
\begin{align*}
\text{Subcase IIC1: } (1 + \mu)(f_1 + m_1) & = 2f_1m_1 \tag{B12}
\end{align*}
\]

or

\[
\begin{align*}
\text{Subcase IIC2: } m_2 & = \frac{2f_2m_1(1 - m_1)}{(1 + \mu)(f_1 + m_1) - 2f_1m_1}. \tag{B13}
\end{align*}
\]

**Subcase IIC1:** Solving (B12) for \( m_i \), we obtain
\[ m_t = \frac{-f_i(1 + \mu)}{1 + \mu - 2f_i} \]  
(B14)

For this solution to be feasible, we must have both \( m_t > 0 \) and \( m_t < 1 \). The former condition requires that the denominator of (B12) is negative, so the latter condition can be expressed as

\[ f_i > \frac{1 + \mu}{1 - \mu}. \]  
(B15)

But as we saw in examining (B5), the right-hand side of this inequality is greater than one. Therefore, this subcase leads to no feasible solutions.

**Subcase IIC2**: Substituting both (B7) and (B13) into (B3), we obtain

Subcase IIC2a:

\[ 2tm_i(1 - m_i) = 1 - \mu \quad \text{and} \quad 2t(1 - m_t) = 1 + \mu \]  
(B16)

or

Subcase IIC2b: \[ f_i = \frac{m_t[1 + \mu - 2t(1 - m_i)]}{1 - \mu - 2tm_i(1 - m_i)} \]  
(B17)

or

Subcase IIC2c: \[ af_i^2 + bf_i + c = 0, \]  
(B18)

where

\[ a = -2t^2m_i^2(1 - \mu)(1 - h) + tm_i(1 - \mu)t(3 + \mu) - s[1 - \mu + 2h(t + \mu)] \]

\[ b = stm_i(1 - \mu^2)(1 - h) - tm_i^2hs[t(1 + \mu)^2 - 2(1 + \mu^2)] \]

\[ - t(1 - \mu)(s + \mu s - 2\mu) \]

\[ - 2m_i[\mu t^2 - (1 - \mu) \]

\[ + s[1 - \mu^2](1 - \mu) \]

\[ - h(2 - 3t + t^2 + \mu^2 - 2\mu^2 + \mu^2t)]. \]

\[ c = t^2m_i^2(1 - \mu)[2 - s(1 + h - \mu + h)] - tm_i^2t(3 - \mu) - s[1 + t + 2\mu - 6\mu t + \mu^2 + \mu^2t] \]

\[ - h(1 + \mu)(2 - 3t + \mu t)] \]

\[ + m_i^2[t^2(1 - \mu)^2 + s(1 + \mu - 2\theta)(1 + \mu - 2\mu\theta) \]

\[ - 2h(1 + \mu)(1 - \theta)(1 + \mu - \theta)]. \]  
(B19)

**Subcase IIC2a**: Combining the two conditions of (B16), we find that

\[ t = \frac{(1 + \mu)^2}{4\mu}, \]  
(B20)

which is clearly greater than one and hence unfeasible.

**Subcase IIC2b**: Substituting (B7), (B13), and (B17) into (B1), we obtain

\[ (1 + \mu)(1 - t) + tm_i(1 - m) = 0 \]  
(B21)

or

\[ d'm_i^2 + b'm_i^2 + c'm_i^2 + d'm_i + e' = 0, \]  
(B22)

where

\[ d' = -2t^2[1 - \mu + 2\mu s - hs(1 + \mu)], \]

\[ b' = 2t(1 - \mu) - s(1 + \mu)^2 - 4\mu t - h(1 + \mu)(2 + \mu - 3t)], \]

\[ c' = -2t(1 - \mu)^2 - s(1 + \mu)^2 - 4t(1 - \mu t + \mu^2) - h[2(1 + \mu)^2 - 9t + 6t^2 - 8\mu t + 6\mu t^2 - 3\mu^2 t]], \]

\[ d' = 4t(1 - \mu)(1 - \theta) + s(1 - \mu)^2(1 + \mu - 2t) \]

\[ - h(3 - 6t + 2t^2 + \mu + 2\mu t - \mu^2 - 2\mu^2 t + \mu^3)], \]

\[ e' = -(1 - \mu)(1 - \theta)[2(1 - \mu) - hs(1 - \mu)] \]  
(B23)

By numerical analysis, at most one root of this equation is biologically feasible over the range 0 < s, t < 1, producing a unique solution for \( m_t \) that corresponds to equilibrium P3 (A/a\(^b \)) polymorphism. As under the maternal case, the easiest way to calculate the other allele frequencies is to repeat the above analysis under the assumption \( f_2 = m_2 = 0 \); this procedure gives the same coefficients as (B23). Both approaches will therefore yield the same solution for \( m_t \), and hence for \( f_1, m_2, \) and \( f_2, \) implying that \( f_1 = m_2 = 0 \). Moreover, we note that equations (B17), (B22), and (B23) are, respectively, identical to (A29), (A30), and (A31) from the maternal inactivation model. Therefore, allele frequencies at equilibrium P3 are identical to those at equilibrium M3.

**Subcase IIC2c**: For the purposes of algebraic tractability, we hereafter assume that \( h = 1/2 \). We then separately examine the cases \( a = b = 0 \) (Subcase IIC2ci), \( a = 0 \neq b \) (Subcase IIC2cii), and \( a \neq 0 \) (Subcase IIC2ciii).

**Subcase IIC2ci** \( (a = b = 0) \): Substituting \( h = 1/2 \) into (B19), setting \( b = 0 \), and solving for \( m_t \), we find that

\[ m_i = 0, \quad m_t = 1, \quad \text{or} \quad m_i = - \frac{2s(1 - \theta) + 2\mu t(2 - s) - 2\mu^2(2t - s)}{st(1 + \mu)(1 - \mu)}. \]  
(B24)

We have already examined the first two solutions; by inspection, the third solution is negative and hence unfeasible.
Subcase IIC2cii (a = 0 ≠ b): Substituting $h = \frac{1}{2}$ into (B19), setting $a = 0$, and solving for $m_i$, we derive

$$m_i = \frac{t(1 + \mu) - s}{t(2 - s)}. \quad (B25)$$

We can then solve (B18) and (B19) for $f_1$ and plug in (B25) to obtain

$$f_i = \left[ t - (1 - 3s) \right] \left[ 4(1 - 2a - \mu^2) - t(5 - 15a + 5a^2 - \mu^2) \right]$$
$$- \frac{3}{4}(3 - 6a - \mu^2 - t(2t - 2a))$$
$$- 8\mu(1 - \mu)(1 - s)(4(1 + \mu^2))$$
$$- \left( t(3 - t - 3a - \mu^2 + \mu^3) \right)$$
$$\left( 3 - t + 7a - 3a^2 + \mu^2 \right)$$
$$- \left[ t(3 - t + 2a + \mu) \right]. \quad (B26)$$

Substituting (B7), (B13), (B25), and (B26) into (B1), we find that

$$4 - 3s + \mu s = 0 \quad (B27)$$

or

Subcase IIC2cii.a: $t + \mu t - s = 0 \quad (B28)$

or

Subcase IIC2cii.b: $4 - 3s + 2\mu t + t^2 = 0. \quad (B29)$

Equation B27 implies that $s = 4/(3 - \mu)$; this value is greater than one and hence unfeasible.

Subcase IIC2cii.a: Substituting back into (B25) and (B26), we obtain $f_1 = m_i = 0$. This case has already been analyzed (Case IIB) and can therefore be excluded from further consideration.

Subcase IIC2cii.b: Solving Equation B29 for $t$, we obtain

$$t = \frac{4 - 3s - \mu s}{3 - 2s - 2\mu - \mu^2}. \quad (B30)$$

Feasibility requires $t < 1$, which implies

$$s > 1 + \mu. \quad (B31)$$

This value of $s$ is greater than one and hence unfeasible. This concludes our analysis of subcase IIC2cii.

Subcase IIC2cii (a ≠ 0): From (B18),

$$f_i = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}. \quad (B32)$$

where the coefficients $a$, $b$, $c$ are given by (B19). We set $h = \frac{1}{2}$, substitute (B7), (B13), and (B32) into (B1), and solve for $m_i$ to obtain

$$4 - 3s + \mu s = 0 \quad (B33)$$

or

Subcase IIC2cii.a: $m_i = \frac{t(1 + \mu) - s}{t(2 - s)}. \quad (B34)$

or

Subcase IIC2cii.b:

$$s[2t(1 - m)(2 + m - \mu m) - t(6 - 3m + 2\mu - \mu^2 m)] + (1 + \mu)[1 - t(1 - m^2)]. \quad (B35)$$

As we saw in examining Equation B27, (B33) is unfeasible, so we can turn to the other two possibilities.

Subcase IIC2cii.a: Substituting (B34) into (B19), we find that $a = 0$. But this contradicts our assumption for Subcase IIC2cii. Therefore, this subcase can be excluded.

Subcase IIC2cii.b: Solving (B35) for $m_i$, we find

$$m_i = \frac{[-s(3 - 2t - 2\mu t + \mu^2)]}{4 s(1 - \mu - s)} \quad (B36)$$

Let us separately analyze the minus root (Subcase IIC2cii.b1) and the plus root (Subcase IIC2cii.b2) of this equation.

Subcase IIC2cii.b1: Feasibility requires $m_i > 0$, which implies

$$s(3 - 2t - 2\mu t + \mu^2) < 0. \quad (B37)$$

Since $s > 0$, this would require

$$t > \frac{3 + \mu^2}{2 + 2\mu}. \quad (B38)$$

This value is greater than one and hence unfeasible.

Subcase IIC2cii.b2: Again, feasibility requires $m_i > 0$, which implies (B37) or

$$16(1 - \mu)(2 - s)(1 - t)(2t + s - 2st - 2\mu t + \mu s) < 0. \quad (B39)$$

We have shown that inequality (B37) is unfeasible. But inequality (B39) implies that

$$t < -\frac{s(1 + \mu)}{2(1 - \mu - s)}. \quad (B40)$$

This value is less than zero and hence also unfeasible. This concludes our analysis of Subcase IIC2c and therefore of model 2 (paternal inactivation).

APPENDIX C: PATERNAL INACTIVATION WITH STABILIZING SELECTION

Our procedures for calculating equilibrium allele frequencies for model 3 closely followed those already outlined in APPENDIX B; we therefore omit a detailed derivation. Equilibrium S1 (fixation of $a^S$) occurs at $f_1 = f_2 = m_1 = m_2 = 0$, $f_3 = m_3 = 1$. Allele frequencies at equilibrium S2 ($a^D/a^S$ polymorphism) are given by

$$f_i = \frac{m_i = 0}{(1 + \mu)(1 - t + 2f_2)}, \quad f_3 = \frac{f_3 + \sqrt{n_3}}{D_3}, \quad (C1)$$
where

\[ D_s = 4t(r + t + \mu \ell), \]

\[ f_s = -(r + 2t - 3\ell t - 4t^2 + 2\mu r + 6\mu t + \mu \ell t - 4\mu t^2 - \mu^2 r), \]

\[ R_s = f_s^2 + 2D_s(1 - \ell)(r + 2t - 4\mu - \mu r + 2\mu \ell). \quad (C2) \]

[A second root, \( f_s = (f_s - \sqrt{R_s})/D_s \), has no feasible solutions.]

At equilibrium S3 (A/a* polymorphism), allele frequencies are given by

\[ f_2 = m_2 = 0, \quad f_i = \frac{m_i[1 + \mu - 2t(1 - m_i)]}{1 - \mu - 2t m_i(1 - m_i)}, \quad a_s m_1^2 + b_s m_1^2 + c_s m_1^2 + d_s m_1 + e_s = 0. \quad (C3) \]

where

\[ a_s = 2t^2(2 - s - 2\mu + 3\mu), \]

\[ b_s = -2t[4t(1 - \mu)(1 - s) + s(1 + \mu)(t - \mu)], \]

\[ c_s = t[s(1 + \mu)(1 + \mu - 2\ell) + 2(1 - \mu)(2 - 2t - 2\mu + 3\mu)], \]

\[ d_s = -8t(1 - \mu)(1 - s) + s(1 - \mu)^3 - 2(1 - \ell)(t + \mu t - 3\mu + \mu^2), \]

\[ e_s = -(1 - \mu)(1 - \ell)(s - 4t + 4\mu - \mu s). \quad (C4) \]

These frequencies are identical to those for equilibrium P3 when \( h = h'_2 \).