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Population Genetic Consequences of the Fragile-X Syndrome, Based on the X-Inactivation Imprinting Model

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Summary

We have examined the population genetic consequences of the model of Laird (Genetics 117:587–599, 1987) in which the fragile-X syndrome is caused by "imprinting" of a mutant chromosome. The imprinting event in this model results from a block to reactivation of an inactive X chromosome prior to oogenesis. If it is assumed that males carrying the imprinted chromosome never reproduce, the frequencies of females and males carrying the imprinted chromosome are expected to be equal. When a mutation-selection balance is established, there are expected to be somewhat more than twice as many females carrying the nonimprinted fragile X as carry the imprinted fragile-X chromosome, the excess depending on the fertility of fragile-X females. Nonpenetrant (transmitting) males, i.e., those with the nonimprinted fragile-X chromosome, are expected to be present at about the same frequency as are males with the syndrome. More than one-third of the nonimprinted chromosomes in the population are expected to be newly arisen in each generation. We have considered possible alternatives to the model of a mutation-selection balance. Nonimprinted carrier females would need to have 100% fertility excess to avoid postulating a high mutation rate to account for the very high prevalence of the syndrome.

Introduction

The human fragile-X syndrome (also called Martin-Bell, or marker-X syndrome [McKusick catalog 30955]; McKusick 1988) has an unusual pattern of inheritance and expression for an X-linked syndrome (Sherman et al. 1985). Especially puzzling is the occurrence of nonpenetrant males who are classified as mentally normal, in contrast to the usually obvious mental retardation described for affected males. The daughters of these nonpenetrant males are obligate carriers of the fragile-X mutation, and yet they, like their fathers, are classified as mentally normal. Mentally impaired grandsons and granddaughters of nonpenetrant males are, however, common. It thus appears that passage of the fragile-X mutation through the germ line of a female is necessary for expression of the phenotype of mental retardation.

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Laird (1987) has postulated that the fragile-X syndrome is a disorder of chromosome imprinting. A DNA mutation, or possibly an epigenetic change, in a normal X chromosome produces an inherited change in the fragile-X region that is not readily detectable by either clinical or cytogenetic phenotype. Males carrying this inherited change are those classified as nonpenetrant (also called "transmitting") males. We will use the genetic notation X^f to describe this nonimprinted state of the mutant fragile-X allele. When X chromosomes bearing this change are inactivated in oogonial cells during the X-inactivation process, the fragile-X mutation is assumed to cause a local block in the reactivation process that normally occurs prior to meiosis. This failure of complete reactivation at the fragile-X site leads to the cytological and phenotypic manifestations of the fragile-X syndrome. The change to the inactivated mutant chromosome is described as "imprinting." The imprinted state of the fragile-X mutant allele will be denoted as X^{f*}. The three states of the fragile-X chromosome region, the genotype notation, and the numbering system are shown in figure 1. Included in this

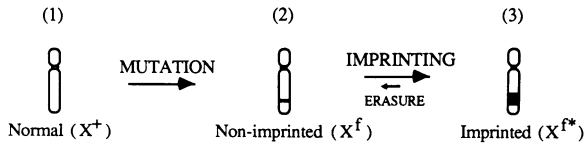

Figure 1 Mutation and imprinting at the fragile-X locus

diagram is the possibility of reversal, or "erasure," of the chromosome imprint.

Throughout the present paper we will use the terms "nonimprinted" and "imprinted" to describe the two states of the mutant fragile-X allele. For brevity, we will sometimes refer to females who carry the nonimprinted mutation as "nonimprinted females" and will refer to other genotypes in a similar manner.

The imprinting hypothesis thus predicts three discrete states of the fragile-X locus—X^+ (i.e., normal), X^f, and X^{f*}, respectively—and a specific probability of one-half for the transition from X^f to X^{f*} in females only. This model makes biological and molecular predictions that are not made by the two-step models proposed by others, in which a "premutation" causes or permits a second genetic defect (Pembrey et al. 1985; Sherman et al. 1985). For population genetic analysis, however, the algebraic formulation of the imprinting model is similar to that considered by Winter (1987) for the "premutation" model, except that we use the specific value of one-half for the transition probability (Laird 1987; Laird et al. 1990), and we consider the possibility of erasure of the imprint in males (Laird, in press).

To approach the population genetic consequences of the X-inactivation imprinting model, we ask the following questions:

1. What are expected to be the relative frequencies of imprinted fragile-X males and females?
2. What are the relative frequencies of non-imprinted and imprinted females?
3. What are the relative frequencies of non-imprinted and imprinted males?
4. What equilibrium is expected if fragile-X mutations are held in the population by a balance between mutation and selection?
5. What is the expected rate of approach to equilibrium?
6. Is there any alternative to a mutation-selection balance to explain the widespread occurrence of the fragile-X condition?

Material and Methods

The parameters for the model, including frequencies in both sexes, rates of mutation, imprinting and erasure, and fertility parameters, are summarized in table 1. The formulation has been simplified by excluding female genotypes with two abnormal chromosomes, on the grounds that these will be very rare. We use the notation of Nagylaki (1977), with Q and q representing the female genotype and chromosome frequencies, respectively. For males, the parameter p suffices for both genotype and chromosome frequencies. The subscripts 1, 2, and 3 are used to refer to the normal, nonimprinted, and imprinted fragile-X alleles, respectively. We have simplified Nagylaki's notation by using Q_1, Q_2, and Q_3—rather than the complete designation Q_{11}, Q_{12}, and Q_{13} for the three female genotypes—since each contains at least one normal allele.

Changes in chromosome frequency over a generation are given in table 2, with the possibility of erasure being omitted for the moment. Sexes are shown separately. Each of the three forces—selection, mutation, and imprinting—affects the two sexes differently.

Selection occurs at the adult (diploid in females) stage (table 2). The fertility of imprinted females is reduced (or conceivably increased) by the amount s. Imprinted males are assumed not to reproduce. Chromosome frequencies are calculated for female gametes, while for males the gamete frequencies are the same as the adult frequencies. Gamete frequencies in the female are expressed in terms of the parameters q_1,q_2, and q_3 from table 1. Mutation is modeled in the usual manner (e.g., see Crow and Kimura 1970, p. 259), with the frequency of the normal X^+ chromosomes in females reduced by the fraction $(1 - u)$ and the excess being added to the nonimprinted X^f class. Similarly, in males the frequency of the X^+ chromosome is reduced by mutation to $p_1(1 - v)$, and the frequency of the X^f chromosome is elevated by the amount $p_1 v$.

According to the model of Laird (1987), the change from X^f to X^{f*}, i.e., the imprinting event, occurs at rate 1/2 in females and does not occur in males. Thus the frequency of X^f chromosomes is reduced from q_2 at birth to $q_2/2$ in gametes, while the frequency of the imprinted X^{f*} class is increased by the same amount, $q_2/2$. Imprinting has no effect on the frequencies of chromosomes in male gametes. The formulation assumes that mutation and imprinting do not occur in the same generation.

Generation equations may now be given. Since the frequencies of the three types of X chromosomes in males are determined only by the female contribution, these frequencies in the next generation—p_1', p_2', and p_3'—may be written, on the basis of the parameter in table 2, as

Table 1

Parameters of the Model, Including Genotype Frequencies at Birth and Rates of Production of Possible Genotypes

A. Genotype

	Females			Males		
	X^+/X^+	X^+/X^f	X^+/X^f	X^+/Y	X^f/Y	X^{f*}/Y
Frequency	Q_1	Q_2	$Q_3/1$	p_1	p_2	$p_3/1$
Selection (reproductive rate)	1	1	$1 - s$	1	1	0
Mutation rate ($X^+ \rightarrow X^f$)	u			v		
Imprinting frequency ($X^f \rightarrow X^{f*}$)		.5			.0	
Erasure frequency ($X^{f*} \rightarrow X^f$)			.0			g

B. Chromosome

	Females			Males		
	X^+	X^f	X^{f*}	X^+	X^f	X^{f*}
Frequency	q_1	q_2	$q_3/1$	p_1	p_2	$p_3/1$
=	$Q_1 + \frac{Q_2}{2} + \frac{Q_3}{2}$	$\frac{Q_2}{2}$	$\frac{Q_3}{2}$			

$$p_1' = \frac{(q_1 - q_3 s)(1-u)}{1 - 2q_3 s}; \tag{1}$$

$$p_2' = \frac{\frac{q_2}{2} + (q_1 - q_3 s)u}{1 - 2q_3 s}; \tag{2}$$

$$p_3' = \frac{\frac{q_2}{2} + q_3(1-s)}{1 - 2q_3 s}. \tag{3}$$

These three equations are not independent, since both left- and right-hand sides sum to unity.

Females and males contribute equally to female progeny (Crow and Kimura 1970, p. 45). Therefore the chromosome frequencies in females may be given by averaging the female and male frequencies from table 2. There are again only two independent equations.

$$q_1' = \frac{(q_1 - q_3 s)(1-u)}{2(1-2q_3 s)} + \frac{p_1(1-v)}{2(1-p_3)}; \tag{4}$$

$$q_2' = \frac{\frac{q_2}{2} + (q_1 - q_3 s)u}{2(1-2q_3 s)} + \frac{p_2 + p_1 v}{2(1-p_3)}; \tag{5}$$

$$q_3' = \frac{\frac{q_2}{2} + q_3(1-s)}{2(1-2q_3 s)}. \tag{6}$$

Inspection of equations (3) and (6) shows immediately that

$$q_3' = \frac{p_3'}{2}. \tag{7}$$

Thus the frequency of the imprinted chromosome in females is at all times expected to be half the frequency in males. This is due to the fact that females transmit this chromosome to male offspring, whereas there is no equivalent transmission of the chromosome from males to female offspring. Since the genotype frequency in fe-

Table 2

Genotype and Chromosome Frequencies for the Fragile-X Mutation with Selection, Mutation, and Imprinting

	A. Females		
	Genotype		
	X^+/X^+	X^+/X^f	X^+/X^{f*}
Frequencies before selection	Q_1	Q_2	Q_3
Frequencies after selection.....	$\frac{Q_1}{1 - Q_3 s}$	$\frac{Q_2}{1 - Q_3 s}$	$\frac{Q_3(1-s)}{1 - Q_3 s}$
	Gamete		
	X^+	X^f	X^{f*}
Frequencies after selection	$\frac{Q_1 + \frac{Q_2}{2} + \frac{Q_3(1-s)}{2}}{1 - Q_3 s}$	$\frac{\frac{Q_2}{2}}{1 - Q_3 s}$	$\frac{\frac{Q_3(1-s)}{2}}{1 - Q_3 s}$
=	$\frac{q_1 - q_3 s}{1 - 2q_3 s}$	$\frac{q_2}{1 - 2q_3 s}$	$\frac{q_3(1-s)}{1 - 2q_3 s}$
After mutation and imprinting	$\frac{(q_2 - q_3 s)(1-u)}{1 - 2q_3 s}$	$\frac{q_2/2 + (q_1 - q_3 s)u}{1 - 2q_3 s}$	$\frac{q_2/2 + q_3(1-s)}{1 - 2q_3 s}$
	B. Males		
	Gamete		
	X^+	X^f	X^{f*}
Frequencies before selection	p_1	p_2	p_3
Frequencies after selection	$\frac{p_1}{(1-p_3)}$	$\frac{p_2}{(1-p_3)}$	0
Frequencies after mutation	$\frac{p_1(1-v)}{(1-p_3)}$	$\frac{(p_2 + p_1 v)}{(1-p_3)}$	0

males is twice the equivalent chromosome frequency (table 1), there is direct equality of genotype frequencies; that is,

$$Q_3' = p_3' . \tag{7a}$$

This result has previously been given by Winter (1987) for the general premutation model.

Equilibrium Frequencies

Under the assumptions we have made, a mutation-selection balance is expected. The equilibrium chromosome frequencies may be obtained by setting $p_1' = p_1 = \hat{p}_1$, $p_2' = p_2 = \hat{p}_2$, $q_1' = q_1 = \hat{q}_1$, etc., in equations (1)–(6). The term $2q_3 s$ in the denominator of equation (6) can be ignored with little loss in accuracy, and the equation then simplifies to

$$\hat{q}_2 = 2\hat{q}_3(1+s) . \tag{8}$$

In terms of genotype frequencies, this equation becomes

$$\hat{Q}_2 = 2\hat{Q}_3(1+s) . \tag{8a}$$

A second important relationship may be found from equations (4) and (1). Equation (4) first simplifies to

$$q_1' = \frac{p_1'}{2} + \frac{p_1(1-v)}{2(1-p_3)} .$$

Writing $p_1' = p_1 = \hat{p}_1$, etc., substituting for p_1 in terms of q_1 from equation (1), setting

$$q_3 = \frac{p_3}{2}$$

from equation (7), and ignoring terms in p_3^2, u^2, p_3u, p_3v, and uv, we find that equation (4) simplifies to

$$\hat{p}_3 = \frac{\hat{q}_1(2u+v)}{\hat{q}1 + 2\hat{q}_1 s - s} .$$

Since $\hat{q}_1$ is close to 1, this can be written with little error as

$$\hat{p}_3 \approx \frac{2u + v}{1 + s} . \qquad (9)$$

It is convenient to define an overall mutation rate u_T, which is weighted to take account of the proportion of the X-linked genes contained in females and males; that is,

$$u_T = \frac{1}{3}(2u+v) .$$

The overall rate as defined in this way serves to determine the equilibrium frequency even if the mutation rate is not the same in females and males. In terms of the overall rate,

$$\hat{p}_3 = \frac{3u_T}{1 + s} \qquad (9a)$$

Thus, in the absence of a selective disadvantage in females, the frequency of the trait in the population follows Haldane's rule for the equilibrium frequency of a sex-linked gene (e.g., see Crow and Kimura 1970, p. 260), with the frequency of the trait in the heterogametic sex, males, being three times the overall mutation rate. The frequency is determined only by the rates of mutation and selection. So long as imprinting occurs, its magnitude does not affect the equilibrium.

The final relationship concerns the frequency of nonimprinted males. When equation (2) is taken into account, equation (5) simplifies to

$$\hat{q}_2 = \frac{\hat{p}_2}{2} + \frac{(\hat{p}_2 + \hat{p}_1 v)}{2(1-\hat{p}_3)}$$

$$= \frac{\hat{p}_2}{2} + \frac{\hat{p}_2(1-v)}{2(1-\hat{p}_3)} + \frac{v}{2} .$$

Approximating the terms $(1-v)$ and $(1-\hat{p}_3)$ by unity reduces the equation to

$$\hat{q}_2 \approx \hat{p}_2 + \frac{v}{2} . \qquad (10)$$

The equilibrium equations (7)–(10) are essentially identical to those derived by Winter (1987). The two treatments differ only in that the equations given by Winter were not normalized for selection coefficients (a difference that disappears when our approximations are made) and in that Winter used the parameter w, which corresponds to our specific value of one-half for the probability of imprinting.

Putting together equations (9), (7a), (8a), and (10), we derive the summary shown in table 3. From this table we can readily see the relationships between each of the four relevant frequencies.

The Possibility of Erasure of Imprinting

When the imprinted fragile-X chromosome is passed through a female, it remains stably imprinted (Laird 1987). The stability of the imprinted state when passed through a male has not been previously addressed. The above analysis has assumed that imprinted fragile-X males do not reproduce at all. It is known, however, that a small fraction, perhaps 1% (Brown et al. 1987), do reproduce. Furthermore, analysis of the published cases of daughters from such fathers suggests that the cytogenetic component of the imprint, and perhaps the clinical component, is efficiently erased in transmission through males (Laird, in press).

We can readily add another parameter to the analysis to account for the possibility of such erasure. We now assume that imprinted fragile-X males reproduce at rate g, instead of rate zero, and that all their transmitted X chromosomes, necessarily in female offspring, have erased the imprint. (A more general treatment would include a variable for the frequency of erasure of the imprint in progeny of imprinted males, taken here to be 1.0. The actual frequency of erasure does not significantly affect the equilibrium values as long as fertility of such imprinted males is low.) The gametes

Table 3

Expected Equilibrium Frequencies for the Four Fragile-X Genotypes

	Female	Male
Nonimprinted	$\hat{Q}_2 = 4u + 2v$	$\hat{p}_2 = 2u + \frac{v}{2}$
Imprinted	$\hat{Q}_3 = \frac{2u + v}{1 + s}$	$\hat{p}_3 = \frac{2u + v}{1 + s}$

Table 4

Expected Equilibrium Frequencies for the Four Fragile-X Genotypes When the Possibility of Erasure in Males at Rate g Is Taken into Account

	Female	Male
Nonimprinted	$\hat{Q}_2 = \frac{(4u+2v)(1+s)}{1+s-g}$	$\hat{p}_2 = \frac{\left(2u+\frac{v}{2}\right)(1+s) - ug}{1+s-g}$
Imprinted	$\hat{Q}_3 = \frac{2u+v}{1+s-g}$	$\hat{p}_3 = \frac{2u+v}{1+s-g}$

produced by males, reflected in the female frequencies of the following generation, are modified slightly to take this into account. The rightmost terms in equations (4) and (5) are replaced by $p_1(1-v)/2[1-p_3(1-g)]$ and $[p_2+p_1v+p_3g]/2[1-p_3(1-g)]$, respectively. The equilibrium frequencies reflecting the modified equations are given in table 4, which is a generalization of table 3. The mutation rate required to maintain the same incidence of fragile-X individuals in the population decreases by the proportion $g/(1+s)$.

The Approach to Equilibrium

Equations (1)–(6) can be iterated by computer to determine the rate of approach to equilibrium in a population. This requires estimates of the parameters u, v, and s. If we assume that the present-day population is at equilibrium, then equation (9a) allows us to estimate the overall mutation rate. We accept a prevalence of fragile-X males of approximately 1/1,000 (Brown et al. 1987). If there is no fertility disadvantage ($s=0$) and equal mutation rates in females and males, then $u = v = 3.3 \times 10^{-4}$. This case is shown graphically in figure 2. The calculation assumes a population that initially contains no abnormal chromosomes, and it follows the increase in frequency until equilibrium. The calculation also assumes no erasure ($g=0$). Note that the figure refers to genotype (rather than chromosome) frequencies, so that the imprinted genotype frequencies are expected to be equal in the two sexes (eq. 7a).

The contribution of newly arisen mutations to the population can be seen by inspection of figure 2. In the initial generation, there is a contribution to the class of nonimprinted males that is equal to the mutation rate. The appearance of imprinted alleles is delayed by a generation. At the equilibrium point, new mutations account for one-third of nonimprinted alleles in the females and for a slightly higher fraction of nonimprinted alleles in the males.

Table 5 shows the effect of assuming both a 20% reduction in female fertility, as estimated by Sherman et al. (1985), and a compensatory increase in mutation to give the same prevalence of fragile-X males. Equation (9a) thus gives a mutation rate of $u = v = 4 \times 10^{-4}$ for this case, when it is assumed that rates in females and males are equal. The results are not very different from those of figure 2. The values of Q_3 and p_3, the frequencies of the imprinted genotypes, are unaltered from the case of $s = 0$, except in the early generations. The values of Q_2 and p_2 at equilibrium are increased by the factor $1 + s$. It should be noted that the equilibrium values depart by a small amount from those predicted by table 3, because of the approximations made in deriving the algebraic values of this table.

The rate of approach to the equilibrium expectations of imprinted to nonimprinted is indicated in the final

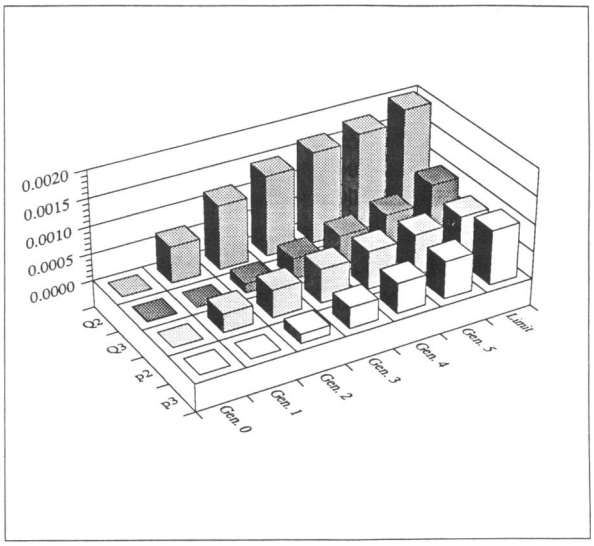

Figure 2 Frequencies of fragile-X genotypes at various generations in a population starting without mutations.

column of table 5. This is calculated from the female frequencies, Q_3/Q_2, normalized by the factor $2(1+s)$ to make the ratio approach unity. Note that this measure does not take into account the absolute frequencies of the two fragile-X genotypes. Initially, it is clear that there are many more nonimprinted than imprinted females in the population. But the ratio takes relatively few generations—fewer than 10—to become indistinguishable from the equilibrium ratio.

The rate of approach to equilibrium appears to be little affected by the assumed magnitude of the mutation rate. Either increasing or decreasing the female and male rates by a factor of 10 leaves the rate of approach to equilibrium almost unchanged. Our computations have also shown that the conclusions are not qualitatively altered by assuming unequal female and male mutation rates or by moderate rates of erasure. The effect of assuming an erasure rate of 1% is shown as the last line of table 5.

Table 5 shows that males carrying the two types of fragile-X chromosomes—i.e., nonpenetrant males and affected males—are expected to occur at about the same frequency at equilibrium. The equality of these two classes is coincidental, depending on the choice of $s = .2$ for the reduction in female fertility. This incidence of nonpenetrant males is clearly much higher than the 20% of affected males estimated by Sherman et al. (1985). There are, however, obvious biases of ascertainment, and Sved and Laird (1988) have shown that a 20% ascertainment probability for nonpenetrant males is consistent with other parameters of the imprinting model.

Alternatives to a Mutation-Selection Balance Model

Some reason must be found for the high frequency of the trait, regardless of whether we assume that the fragile-X syndrome is at equilibrium. We have investigated models of selection in which females carrying either the nonimprinted or the imprinted fragile-X chromosome have increased fertility compared with females carrying the normal X chromosome. The principal motivation is to see whether models can be devised to obviate the need for high levels of mutation in accounting for the observed prevalence of the trait.

It seems easy to rule out models of increased fertility of females carrying imprinted alleles. Under these conditions, nonimprinted chromosomes would be systematically lost from the population through imprinting and would not be replenished by mutation. Also, when one puts $q_2 = 0$ into equation (6), it can be seen that in order to oppose the loss of imprinted fragile-X chromosomes from the male, imprinted fragile-X females would need to have double the fertility of normal females, i.e., $s = -1$. There is some suggestion that females classified as mildly retarded may have increased fertility, although it seems doubtful that the increased fertility of these females is sufficient to overcome the

Table 5

Chromosome Frequencies Expected with Selection against Imprinted Fragile-X Females ($u = v = .0004$, $s = .2000$)

	Female		Male		
Generation	X^+/X^f Q_2	X^+/X^{f*} Q_3	X^f/Y p_2	X^{f*}/Y p_3	$2(1+s)Q_3/Q_2$
0	.000000	.000000	.000000	.000000	—
1	.000800	.000000	.000400	.000000	.00
2	.001400	.000200	.000600	.000200	.34
3	.001749	.000430	.000750	.000430	.59
4	.001987	.000609	.000837	.000609	.74
5	.002133	.000740	.000896	.000740	.83
6	.002229	.000830	.000933	.000830	.89
7	.002290	.000889	.000957	.000889	.93
8	.002329	.000928	.000972	.000928	.96
9	.002354	.000954	.000982	.000954	.97
10	.002370	.000970	.000988	.000970	.98
15	.002396	.000997	.000998	.000997	1.00
20	.002399	.001000	.000999	.001000	1.00
∞	.002400	.001000	.000999	.001000	1.00
∞($g = .01$)	.002419	.001008	.001004	.001008	

reduced fertility of severely affected females (Sherman et al. 1984, table 8). The possibility of a doubling of fertility seems remote.

The second possibility is that of increased fertility of females carrying the nonimprinted fragile-X mutation. This situation is more easily visualized in terms of a flow diagram (fig. 3). This figure shows also the dynamics of the system when new mutations are being continually produced. In the absence of new mutations, it is clear that the pool of nonimprinted mutations in females will be halved in each generation, because of imprinting. Nonimprinted females would thus need to have double the fertility of normal females in order to oppose this loss and maintain an equilibrium frequency of nonimprinted mutations. Since the nonimprinted chromosome is passed on intact by nonpenetrant males, an equilibrium could also be produced with a combined fertility excess in nonpenetrant males and in nonimprinted females. The magnitude of fertility excess required in females would then be less than 100%, but it would still be sufficient to make this an unlikely model. Our conclusion from the analysis is therefore similar to that of Vogel (1984)—i.e., that very high levels of fertility excess need to be proposed to accommodate a reduced mutation rate.

It is also questionable whether a stable equilibrium could be produced by a fertility excess. The net effect would be to cancel out the different selective forces, leading to what may be described as a "neutral equilibrium." Any departure from such an equilibrium would not be opposed by a selective force tending to restore the equilibrium.

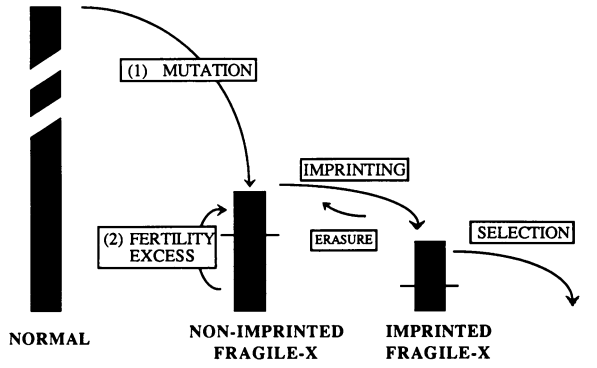

Figure 3 Diagram indicating the dynamics of the fragile-X system when either de novo production of nonimprinted mutations (1) or no new mutations but a fertility excess of nonimprinted females (2) is assumed.

Discussion

The results of the population genetic calculations may be summarized in the six conclusions below. The first five of these conclusions are illustrated by table 3 and by the numerical examples of figure 2 and table 5.

1. The frequency of the imprinted fragile-X chromosome is expected to be twice as high in males as in females. When the fact that females have two X chromosomes is taken into account, the frequency of imprinted fragile-X females is expected to be equal to the frequency of imprinted fragile-X males.

2. At equilibrium, females with the nonimprinted and imprinted fragile-X chromosomes are expected in the ratio $2(1+s):1$, where $1 - s$ is the relative fitness of female carriers of the imprinted fragile X.

3. The equilibrium numbers of transmitting and affected males depend on the ratio of mutation rates in the two sexes and on the relative fitness of female carriers of the imprinted fragile X. When a reduction in fitness of 20% for imprinted female carriers is assumed, the ratio of transmitting to affected males ranges from 0.625 if all mutation occurs in males, through 1.0 for equal mutation rates in the two sexes, to 1.25 if all mutation occurs in females (table 3, col. 2).

4. The equilibrium frequency of the imprinted allele is $(2u+v)/(1+s)$, where u and v are the mutation rates in females and males, respectively. With a prevalence of 10^{-3} for affected males, $u = v$, and $s = 0.2$, the required mutation rate is $u = 4 \times 10^{-4}$ in the absence of erasure of the imprint. Equivalently, about one-third of mutations are newly arisen in each generation. The mutation rate required to maintain equilibrium is reduced in proportion to the reproductive rate of imprinted males, if efficient erasure of the imprint in progeny of such males is assumed. Sherman et al. (1984, 1985) have previously commented on the very high rate of new mutations implied by the mutation-selection balance.

5. The rate of approach to equilibrium is reasonably rapid, so that near-equilibrium conditions are expected to be reached approximately 5–10 generations after the trait first appears in a population. Thus it cannot be ruled out that an increase to a high mutation rate occurred as recently as 100–200 years ago.

6. Any model in which the fragile-X mutation is not continually replenished by a supply of new mutations requires a fertility excess for carriers of the mutation of close to 100% and is therefore unlikely.

The expected equality of imprinted fragile-X female and male frequencies (conclusion 1) is an intuitively clear result. It follows simply from the fact that males do not pass on the imprinted fragile-X chromosome.

Males and females carrying the imprinted fragile X differ only in that the former get a Y chromosome from the father and the latter a normal X chromosome. Note that this result would not be true under a model in which the imprinted fragile X is produced in a single mutational step in males.

Conclusions 2 and 3 make predictions regarding the equilibrium frequencies of nonimprinted and imprinted genotypes in the population. These predictions are of course difficult to test, since imprinted genotypes are ascertained at a much higher rate than are nonimprinted genotypes. Elsewhere we examine models in which variable ascertainment probabilities are taken into account. Our conclusion is that, for realistic ascertainment probabilities, the observed population frequencies are consistent with the equilibrium predictions of the X-inactivation imprinting model (Sved and Laird 1988).

Finally, it is of interest to consider further the reasons for the high prevalence of the fragile-X syndrome. In conclusion 6, we argue that a constant supply of new mutations is a more realistic model than is a fertility-excess model. Holmgren et al. (1988), however, showed that a number of apparently unrelated cases of fragile X in northern Sweden could be traced genealogically to a probable single ancestral mutation. The mutation-selection balance model would predict that pedigrees that stretch back for many generations will not be common. It is possible that, in a period of population expansion, the frequency of the fragile-X chromosome has increased by chance. Such an explanation could not, however, account for a systematic rise of the mutation in many different populations. All that can be argued in a case where the prevalence of the syndrome has increased by chance is that a lower equilibrium frequency—and therefore a lower mutation rate—may be appropriate.

It is premature to attempt a definite answer to the question of high prevalence. Characterization of DNA at or near the fragile-X site can be expected at some time in the future. We would anticipate that data on linkage disequilibrium with very closely linked DNA markers (RFLPs) will then provide a clearer answer to the question of whether there have been numerous recent origins for the mutation, as expected on the basis of the analysis presented in the present paper, or whether there has been long-term survival of a few mutations.

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