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Population genetic aspects of deleterious cytoplasmic genomes and their effect on the evolution of sexual reproduction

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Summary

A conflict of interest may arise between intra-cellular genomes and their host cell. The example explicitly investigated is that of a 'selfish' mitochondrion which increases its own rate of replication at the cost of reduced metabolic activity which is deleterious to the host cell. The results apply to deleterious cytoplasmic agents in general, such as intracellular parasites. Numerical simulation suggests that selfish mitochondria are able to invade an isogamous sexual population and are capable of reducing its fitness to below 5% of that prior to their invasion. Their spread is enhanced by decreasing the number of mitotic divisions between meioses, and this may constitute a significant constraint on the evolution of lifecycles. The presence of such deleterious cytoplasmic agents favours a nuclear mutation whose expression prevents cytoplasm from the other gamete entering the zygote at fertilization, resulting in uniparental inheritance of cytoplasm. Such a mutation appears physiologically plausible and can increase in frequency despite its deleterious effect in halving the amount of cytoplasm in the zygote. It is suggested that these were the conditions under which anisogamy evolved. These results have implications for the evolution of sexual reproduction. Standard theory suggests there is no immediate cost of sex, a twofold cost being incurred later as anisogamy evolves. The analysis described here predicts a large, *rapid* reduction in fitness associated with isogamous sexual reproduction, due to the spread of deleterious cytoplasmic agents with fitness only subsequently rising to a *maximum* twofold cost as uniparental inheritance of cytoplasm and anisogamy evolve.

1. Introduction

Sexual reproduction is one of the most widespread features of genetic systems and one of the more problematic subjects in evolutionary biology. In particular, much effort has been expended trying to explain how its maintenance can compensate for the well known 'twofold cost of sexual reproduction'. In reality this twofold cost is not an inherent cost of sex but is due to the uniparental inheritance of cytoplasm. This is commonly associated with *anisogamy*, the production of gametes differing in size within a population. The alternative strategy, *isogamy*, where all gametes are identical in size and function avoids this cost (Maynard-Smith, 1989: 237–8; appendix, this paper); implicit in this definition of isogamy is that both gametes make equal contributions of cytoplasm to the zygote, i.e. there is biparental inheritance of cytoplasm. The adoption of anisogamy by higher organisms had profound effects on their subsequent evolution as it formed the basis for sexual selection. Previous theories on the evolution of anisogamy have assumed that disruptive selection of gamete size resulted from two conflicting strategies of producing a large number of small gametes or a small number of large gametes (Parker *et al.* 1972; Bell, 1978; Maynard-Smith, 1978). The following account is fundamentally different: it postulates isogamy to be disadvantageous as it allows deleterious cytoplasmic organisms (such as intracellular parasites) to spread through a population when cytoplasms are shared at fertilization. Conversely, uniparental inheritance restricts deleterious genomes to single cytoplasmic lineages and they cannot spread through a population. It will subsequently be argued that once uniparental inheritance is established, the ('male') gametes which contribute no cytoplasm may become small and anisogamy may evolve.

This study therefore has three main objectives. Firstly, to investigate the population genetics of deleterious cytoplasmic genomes and identify the constraints they may impose on the evolution of isogamous sexual life-cycles (for example, their spread appears to be inhibited by lifecycles with small gametes

or a large number of mitotic cell divisions between meioses). Secondly, to determine whether the presence of such genomes is likely to reduce the mean fitness of an isogamous population below 0·5; in this situation uniparental inheritance of cytoplasm will be more fit despite its inherent twofold cost. Thirdly, to extend this group selectionist argument to selection on individual genes by showing that a gene encoding uniparental inheritance of cytoplasm can invade an isogamous population in the presence of deleterious cytoplasmic agents.

This study adapts the methodology previously developed as a general model of non-mendelian genetics (Hastings, 1991). It allows numerical investigation of the population genetics of cytoplasmic genomes and provides a quantitative basis for the discussions of Grun (1976), Eberhard (1980) and Cosmides & Tooby (1981). The model will explicitly investigate the population dynamics of 'selfish' mitochondria. These mitochondria increase their own rate of replication at the cost of a reduced metabolic activity which is deleterious to the host cell. Mitochondria were chosen as they are ubiquitous in eukaryotes and essential for viability; furthermore, an inherent conflict may arise between the alternatives of exporting the energy produced during oxidative phosphorylation for general cellular metabolism, or sequestering it to increase their own rate of replication. However, as will be discussed later, the results are applicable to deleterious cytoplasmic agents in general.

2. Description of model

The simulation model is similar to those described by Chapman *et al.* (1982) and Takahata & Slatkin (1983). A generalized life-cycle of organisms with non-overlapping generations is shown in Fig. 1. The life-cycle is assumed to start with zygotes which undergo a number of mitotic divisions prior to gametogenesis. Meiosis may occur at position (1) or (2) on Fig. 1 resulting in the life-cycle being predominantly haploid or diploid respectively; the model is applicable to both.

A key feature occurs during mitosis when daughter cells inherit mitochondria *sampled* from those present in the parental cell at the time of division. This sampling creates diversity in the mitotic offspring derived from each zygote; for example a cell containing 100 mitochondria of which two are mutants will produce daughter cells each of which initially contains 50 mitochondria with either zero, one,or two mutants with frequencies of 0·25, 0·5 and 0·25 respectively (this occurs if sampling is without replacement and serves to illustrate the general principle that diversity is created by sampling). In the model developed here, sampling is with replacement, i.e. if a cell contains n mitochondria each daughter cell contains n mitochondria sampled with replacement as described in more detail later. Sampling with replacement was preferred for two reasons. Firstly, it is mathematically simpler than sampling half the cellular number of mitochondria as this necessitates an additional growth phase (this method was also used and the results were virtually identical to those obtained by sampling with replacement). Secondly, replication of mitochondria is usually faster than the rate of cell division, resulting in sampling differences in replication between cell divisions; sampling with replacement models these processes and is therefore more realistic (C. W. Birkey, pers. comm.). This diversity allows two opposing processes to operate: firstly, selfish mitochondria will increase in 'infected' cells due to their higher rate of replication and, secondly, selection will favour those cells containing fewest selfish mitochondria. A critical element of the model is therefore to calculate the distribution of cytotypes after the mitotic divisions and prior to gametogenesis (an example is shown on Fig. 2, see later). The mitotic divisions may occur in free-living cells, such as yeasts, or within the germline of a metazoan; the model is applicable to both. The mitochondria in gametes are sampled from post-mitotic cells and subsequent random fusion of gametes produces fertilized cells.

A single nuclear gene with two alleles U (uniparental) and B (biparental) is assumed to determine the behaviour of gametes when they combine to produce a zygote: a zygote formed from two B-bearing gametes inherits haploid genomes and cytoplasms from both gametes; a zygote formed from a U-bearing gamete and a B-bearing gamete inherits haploid genomes from both gametes but its cytoplasm is derived only from that of the U-bearing gamete; a zygote formed from two U-bearing gametes inherits haploid genomes from both gametes but its cytoplasm is derived from only one (assumed to be equally likely to inherit either cytoplasm). The latter two situations are examples of the familiar uni-parental inheritance of cytoplasm which exists in most higher organisms. This assumes that gamete behaviour at fusion is determined by the haploid genotype; in many extant species, for example mammals, gamete behaviour (and morphology) is determined by the diploid genotype and the dominance relationship between U and B will be considered later. The lifecycle is assumed to initially contain only B alleles, with mutation to the U allele being simulated later. Selection on the basis of cytotypes is assumed to act on zygotes and their mitotic offspring, but not on gametes.

It is assumed that two types of mitochondria are present in the population: the wild type (w.t.), and selfish types. A selfish mitochondrion has two properties, its metabolic activity, a, and its rate of replication, r, both expressed relative to w.t. whose value is unity. Each cell is assumed to contain n mitochondria resulting in $n+1$ potential cytotypes. In the matrix notation developed hereafter, a cytotype containing 0 selfish mitochondria is referenced by element $i = 0$, a cytotype with 1 selfish mitochondria by $i = 1$ and so

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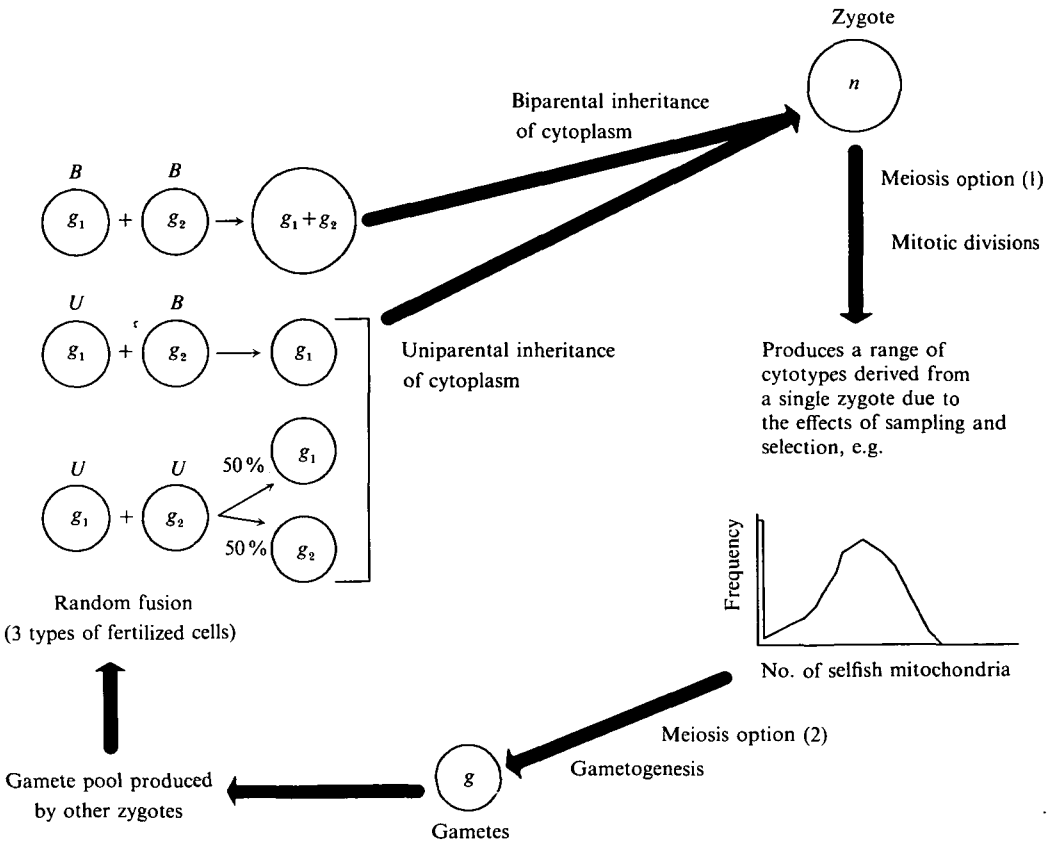

Fig. 1. Life-cycle of a model organism. The life-cycle may be predominantly haploid or diploid depending on whether meiosis occurs at position (1) or (2) respectively. The numbers inside cells (n or g) refer to the number of mitochondria in the cell; at fertilization these are designated g_1 and g_2 to demonstrate whether cytoplasmic inheritance is uni- or biparental (details in text). The nuclear alleles U and B undergo normal mendelian segregation and inheritance.

on. A $(n+1)\times(n+1)$ matrix W can be constructed whose off-diagonal elements are zero and whose diagonal elements $\mathbf{W}_{ii}$ hold the fitness of a cytotype containing i selfish mitochondria. The fitness of a cell is assumed to be proportional to the total activity of its mitochondria hence:

$$\mathbf{W}_{ii} = (ia+n-i)/n.$$

It is now possible to consider each of the stages of the life-cycle in turn before generating a model of the entire life-cycle.

(i) *Mitotic divisions*

A $(n+1)\times(n+1)$ matrix $\mathbf{S}$ is constructed whose elements i,j hold the probability of a cytotype containing j selfish mitochondria being sampled with replacement from a parent cytotype containing i selfish mitochondria during mitosis hence:

$$\mathbf{S}_{ij} = \binom{n}{j}[ri/(ri+n-i)]^{j}[(n-i)/(ri+n-i)]^{n-j}, \qquad (1)$$

where

$$\binom{n}{j} = \frac{n!}{j!(n-j)!}.$$

This formula is not appropriate when only a single type of mitochondria is present in the parent cell, so there are two exceptional elements: $S_{0,0} = 1{\cdot}0$ and $S_{n,n} = 1{\cdot}0$. The higher rate of replication, r, of selfish mitochondria is simulated by a higher probability of their being sampled so the number of selfish mitochondria, i, is scaled by r in the second term of equation (1).

A $(n+1)$ element row vector $\mathbf{F}$ holds the *relative* frequencies of the cytotypes prior to mitosis. $\mathbf{F}_0$ holds the relative frequencies of cells at generation zero, and $\mathbf{F}_m$ the relative frequencies after m mitotic divisions. Hence

$$\mathbf{F}_m = \mathbf{F}_0(\mathbf{SW})^m. \qquad (2)$$

In practice all elements of $\mathbf{F}_0$ are set to zero except the one corresponding to the desired zygote cytotype $0 \leqslant i \leqslant n$ which is set to unity. Equation (2) therefore gives the distribution of cytotypes produced from a single zygote immediately prior to gametogenesis.

(ii) *Gametogenesis*

If each gamete contains g mitochondria, a $(n+1)\times(g+1)$ matrix $\mathbf{T}$ can be constructed whose elements i,j hold the probability of a gamete cytotype

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containing j selfish mitochondria being sampled with replacement from a parental cell containing i selfish mitochondria, hence

$$\mathbf{T}_{ij} = \binom{g}{j}[ri/(ri+n-i)]^j[(n-i)/(ri+n-i)]^{g-j},$$

with, as before, two exceptional elements $\mathbf{T}_{0,0} = 1{\cdot}0$ and $\mathbf{T}_{n,g} = 1{\cdot}0$.

Each possible zygote cytotype can be investigated in turn by setting its frequency to unity in $\mathbf{F}_0$, the distribution of its daughter cells after m mitotic divisions obtained from equation (2) and its gametic output is given by $\mathbf{F}_m\mathbf{T}$. The relative frequencies of gametes produced from each zygote are scaled to sum to unity and a matrix $\mathbf{G}$ constructed whose elements i, j hold the frequency of gametes containing j selfish mitochondria being produced after m mitotic divisions from an original zygote containing i selfish mitochondria. The matrix $\mathbf{G}$ therefore describes the mitotic stage of the life-cycle in terms of the gametic output from each zygote cytotype, and can be used when modelling the entire life-cycle.

(iii) *Fertilization*

A $(n+1)$ element row vector $\mathbf{A}_t$ holds the frequency of zygotes in the population at generation t. The relative frequencies of gametes produced by this population after m mitotic divisions is given by:

$$\mathbf{A}_t\mathbf{WG}. \tag{3}$$

The relative frequencies of fertilized cells in generation $t+1$ is calculated assuming random fusion of gametes. When g is less than $n/2$ the number of mitochondria in the zygote must increase from $2g$ to n prior to mitosis; this is achieved by sampling n from $2g$ with replacement, and the probability of selecting j selfish and $n-$j w.t. mitochondria is

$$\binom{n}{j}[ri/(ri+2g-i)]^j[(2g-i)/(ri+2g-i)]^{n-j}, \tag{4}$$

where i is the number of selfish mitochondria in the cytoplasm of the fertilized cell.

The relative frequencies of zygotes are normalized thereby completing a generation. The generations were iterated until the proportional change in the frequency of selfish mitochondria was less than 10^{-8} over a 10 generation interval, when equilibrium was assumed to have been reached.

(iv) *Introduction of the* U *allele*

When equilibrium frequencies of selfish and w.t. mitochondria have been reached, a mutation to the U allele is simulated by converting a proportion 10^{-8} of gametes of cytotype i to U-bearing gametes of cytotype i (for reasons discussed later the cytotype i is not important). U-bearing zygotes inherit only half the normal amount of cytoplasm, which may result in reduced fitness, and their relative fitness is denoted ω. The models examined will assume $\omega = 0{\cdot}5$ as this is the simplest case and assumes fitness is proportional to zygote volume. In zygotes formed from a U-bearing gamete, growth in mitochondrial number prior to mitosis is from g to n; as before this is achieved by sampling as in equation (4) except that the term $2g$ is replaced by g. Introduction of the U allele requires three vectors of type $\mathbf{A}$ to track the three genotypes: $\mathbf{A}_{UU}$, $\mathbf{A}_{UB}$ and $\mathbf{A}_{BB}$ where the subscript refers to the genotype. The gametic output of the three genotypes is:

$$\mathbf{A}_{UU}(\omega\mathbf{W})\mathbf{G}; \quad \mathbf{A}_{UB}(\omega\mathbf{W})\mathbf{G}; \quad \mathbf{A}_{BB}\mathbf{WG},$$

respectively; analogous to equation (3) but with the addition of the scalar ω representing the reduction in fitness due to decreased zygote volume in genotypes UB and UU. The genotypes and cytotypes of zygotes in the subsequent generation are calculated assuming random fusion of gametes and normalizing so that their frequencies sum to unity. The life-cycle was iterated until equilibrium frequencies of the two types of mitochondria and three genotypes was reached.

The model can be extended to examine 'paternal leakage', the situation where most cytoplasm is inherited from one 'maternal' gamete with a much smaller contribution from the 'paternal' gamete. Matrix $\mathbf{T}$ can be altered to sample the appropriate number of mitochondria in 'maternal' and 'paternal' gametes and used to generate the corresponding $\mathbf{G}$ matrices. In U/B fusions the U-bearing gamete makes the maternal contribution, and the B-bearing gamete the paternal contribution; in U/U fusions each gamete has an equal probability of making the maternal contribution. In all other respects the model is unchanged.

(v) *Models investigated*

In all models investigated the number of mitochondria per cell, n, was set to 100. The model was initialized by setting all elements of $\mathbf{A}_0$ to zero except the elements representing cytotypes with 100 w.t. mitochondria (set to $1-10^{-8}$) and 99 w.t. mitochondria (set to 10^{-8}); the fraction 10^{-8} cells with a single selfish mitochondrion represents a single mutation in a finite population. Two models are investigated: a 'budding yeast' model where gametes are assumed to contain 5 mitochondria, and a 'metazoan' model where the gamete is produced with half the cellular complement of mitochondria, i.e. 50 mitochondria per gamete. The model can be extended to deleterious cytoplasmic organisms in general by altering the definition of some of the parameters. The replication rate, r, remains the same but the metabolic activity, a, becomes a measure

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of the pathogenicity of the organism, for example $a = 1$ means it is completely non-pathogenic, whereas $a = 0$ means it is potentially lethal. The number of mitochondria in the cell or gamete becomes the maximum permissible number of deleterious genomes (i.e. the maximum level of infection), and a w.t. mitochondrion is equivalent to an uninfected portion of cytoplasm.

The model was also run with the following parameters: (i) 50 mitochondria in zygotes and mitotic cells with 3 or 25 mitochondria per gamete, (ii) 500 mitochondria in zygotes and mitotic cells with 25 or 250 mitochondria per gamete. The results were qualitatively similar to those presented later and are omitted for brevity.

3. Results

A range of replication rates and metabolic activities were investigated. The number of mitotic divisions between gametogenesis appears to be a critical factor limiting the spread of selfish mitochondria (Table 1). Changes in the frequency of cytotypes and selfish mitochondria during mitosis are shown on Fig. 2 for $r = 1{\cdot}2$ and $a = 0{\cdot}2$. The dynamics are due to the interaction of two processes. First, the replicative advantage of selfish mitochondria increases their frequency in 'infected' cell lineages (Fig. 2*a*). Second, sampling creates cell lineages without selfish mitochondria and because these are the fittest type of cell they increase at the expense of 'infected' cell lineages and eventually dominate the population (Fig. 2*b*).

Table 1. *The maximum number of mitotic divisions in the life-cycle which allow selfish mitochondria to invade a population. The symbol '—' indicates that selfish mitochondria are unable to invade even when no mitotic divisions occur*

(*a*) 5 mitochondria per gamete

	Reproductive rate					
Activity	1·01	1·05	1·1	1·2	1·8	2·0
0	—	—	7	15	1	—
0·05	—	—	8	16	1	—
0·2	—	—	12	19	4	1
0·5	—	11	23	35	108	131
0·8	—	36	58	95	344	421

(*b*) 50 mitochondria per gamete

	Reproductive rate					
Activity	1·01	1·05	1·1	1·2	1·8	2·0
0	—	6	11	16	18	19
0·05	—	6	12	16	23	26
0·2	—	8	14	19	39	46
0·5	—	13	22	32	89	105
0·8	7	35	55	89	275	326

The overall frequency of selfish mitochondria is determined by the interaction of these two processes: their frequency initially rises due to their replicative advantage but eventually falls as cytoplasms without selfish mitochondria dominate the population (Fig. 2*b*). This explains why the number of mitotic divisions limits the ability of selfish mitochondria to invade a population.

The phenomenon whereby a cytotype initially containing a mixture of selfish and w.t. mitochondria will eventually (given enough mitotic divisions) produce cytoplasms containing either exclusively w.t. or exclusively selfish mitochondria is known as 'mitotic segregation' and has been demonstrated in yeast (Alberts *et al.* 1983, p. 533). In mathematical terms this occurs since equation (2) is a Markov chain with two absorbing states corresponding to cytotypes containing a single type of mitochondrion. If the nuclear genome contains a U allele, mitotic segregation may result in it being associated with a cytoplasm containing exclusively w.t. mitochondria; this allele excludes cytoplasm (and hence mitochondria) from the other gamete at fertilization so becomes effectively 'linked' to a cytoplasm containing only w.t. mitochondria, with important consequences for its subsequent dynamics (see later).

Table 2 shows examples of the equilibrium frequencies of selfish mitochondria when present in a population and their effect on fitness. Invasion can occur over a broad range of replication rates and activities and it appears to be a robust conclusion that such organisms can cause a significant reduction in the fitness of isogamous sexual populations. For any value of a there appears to be an optimum value of r (e.g. $a = 0$, 0·05 or 0·2; Table 1*a*); below this level the reproductive advantage of selfish mitochondria is too low for them to spread across mitotic clones, and above this value they multiply so rapidly that clones containing selfish mitochondria rapidly become unfit and are eliminated from the population. This is analogous to the dynamics of parasite systems where the reproductive benefits of increased infection are balanced by decreased fertility or viability of the host (Anderson & May, 1979; May & Anderson, 1979).

Successful invasion of the U allele is due to mitotic segregation producing a U allele 'linked' to a cytoplasm containing only w.t. mitochondria. The only fitness cost incurred by such a combination is due to its excluding cytoplasm from the other gamete at fertilization, and it therefore has fitness ω. If the presence of selfish mitochondria in the population has reduced its mean fitness below ω, the U allele will spread through the population. The mean fitness of zygotes and the frequency of selfish mitochondria at equilibrium before and after introduction of the U allele are shown on Table 3. After introduction of the U allele, equilibrium frequencies of U and B are reached when the fitness of the U allele associated with a cytotype of purely w.t. mitochondria (ω) is exactly

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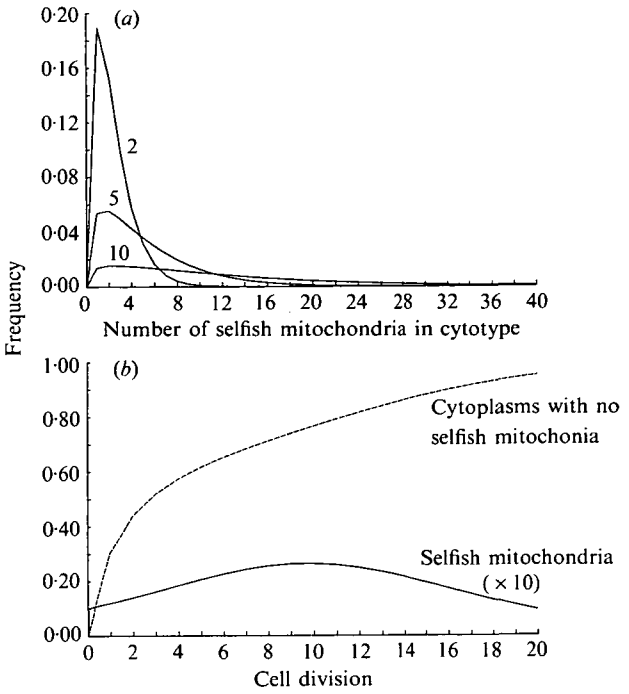

Fig. 2. An example of changes in the relative frequencies of cytotypes and selfish mitochondria in the mitotic offspring of a zygote containing a single selfish mitochondria with relative metabolic activity $a = 0{\cdot}2$ and replicative advantage $r = 1{\cdot}2$. (*a*) The normalized distribution of cytotypes after 2, 5, and 10 mitotic cycles omitting the cytotype class containing no selfish mitochondria. (*b*) The frequencies of cytoplasms containing no selfish mitochondria and the overall frequency of selfish mitochondria.

Table 2. *The frequency of selfish mitochondria in zygotes (f_{mit}) and the mean fitness of zygotes ($\bar{w}$) at equilibrium for a range of replication rates and activities when 10 mitotic divisions occur in the life-cycle and 50 mitochondria are present in each gamete*

		Reproductive rate (r)				
Activity	Parameter	1·05	1·2	1·5	2·0	5·0
0·0	f_{mit}	0·00	0·78	0·95	0·98	1·00
0·0	$\bar{w}$	1·00	0·22	0·05	0·02	0·00
0·05	f_{mit}	0·00	0·84	1·00	1·00	1·00
0·05	$\bar{w}$	1·00	0·20	0·05	0·05	0·05
0·2	f_{mit}	0·00	1·00	1·00	1·00	1·00
0·2	$\bar{w}$	1·00	0·20	0·20	0·20	0·20
0·5	f_{mit}	0·90	1·00	1·00	1·00	1·00
0·5	$\bar{w}$	0·55	0·50	0·50	0·50	0·50
0·8	f_{mit}	1·00	1·00	1·00	1·00	1·00
0·8	$\bar{w}$	0·80	0·80	0·80	0·80	0·80

that of the mean fitness of the *B* allele. The fitness of *B* alleles is determined by the proportion of selfish mitochondria in their cytotypes, so for any given value of ω an increase in metabolic activity results in a higher equilibrium frequency of selfish mitochondria; hence in Table 3, as a increases, so does f'_{mit}.

Table 3. *Equilibrium values of several population parameters obtained when $r = 1{\cdot}2$, $\omega = 0{\cdot}05$, and 10 mitotic divisions occur between meiosis*

	5 mitochondria per gamete			50 mitochondria per gamete		
Activity...	0·0	0·05	0·2*	0·0	0·05	0·2
f_{mit}	0·76	0·84	1·00	0·78	0·84	1·00
$\bar{w}$	0·24	0·21	0·20	0·22	0·20	0·20
f'_{mit}	0·42	0·43	0·48	0·40	0·41	0·48
f_U	0·08	0·09	0·12	0·11	0·12	0·12
$\bar{w}'$	0·5	0·5	0·5	0·5	0·5	0·5
f_{U_1}	—	—	—	0·05	0·05	0·05
$\bar{w}'_1$	—	—	—	0·39	0·38	0·34
f_{U_2}	—	—	—	0·03	0·03	0·004
$\bar{w}'_2$	—	—	—	0·30	0·28	0·21

* The dynamics of this combination of parameters are shown in Fig. 1.

f_{mit} is the frequency of selfish mitochondria before introduction of the *U* allele, $\bar{w}$ is the mean fitness of zygotes prior to introduction of the *U* allele, f'_{mit} is the frequency of selfish mitochondria after introduction of the *U* allele, f_U is the equilibrium frequency of the *U* allele, and $\bar{w}'$ is the mean fitness of the population after introduction of the *U* allele. Also shown is the frequency of the *U* allele and mean fitness of the population when there are 50 mitochondria per gamete and paternal leakage of one mitochondrion (f_{U_1} and $\bar{w}'_1$) and two mitochondria (f_{U_2} and $\bar{w}'_2$), corresponding to leakage rates of 2 and 4% respectively.

If the *U* allele does not result in completely uniparental inheritance of cytoplasm, that is 'paternal leakage' is present, the frequency of the *U* allele may fall dramatically (Table 3) as it cannot become 'linked' to a cytoplasm containing only w.t. mitochondria. The effects of paternal leakage are sensitive to the parameters used and will be discussed later, in the meantime it will be ignored.

After the *U* allele has become linked to a cytoplasm containing only w.t. mitochondria, zygotes of genotype *UB* contain exclusively w.t. mitochondria and consequently produce *B*-bearing gametes with cytoplasms containing only w.t. mitochondria, an effect hereafter called 'cyto-conversion'. These *B* alleles are relatively fit because they have few selfish mitochondria in their cytoplasm and do not incur the cost ω if they fertilize other *B*-bearing gametes; therefore they initially increase in frequency. Selection eventually eliminates them when the number of selfish mitochondria in their cytoplasms (introduced during fusions with *B* gametes containing selfish mitochondria) have increased to the extent that their fitness falls below ω; this introduces a time lag into the dynamics. The distribution of cytotypes associated with *B* alleles is determined by the complicated dynamics between cyto-conversion (determined by the frequency of the *U* allele) and subsequent selection, and may explain the counter-intuitive observation that as a increases the frequency of *B* decreases (Table 3).

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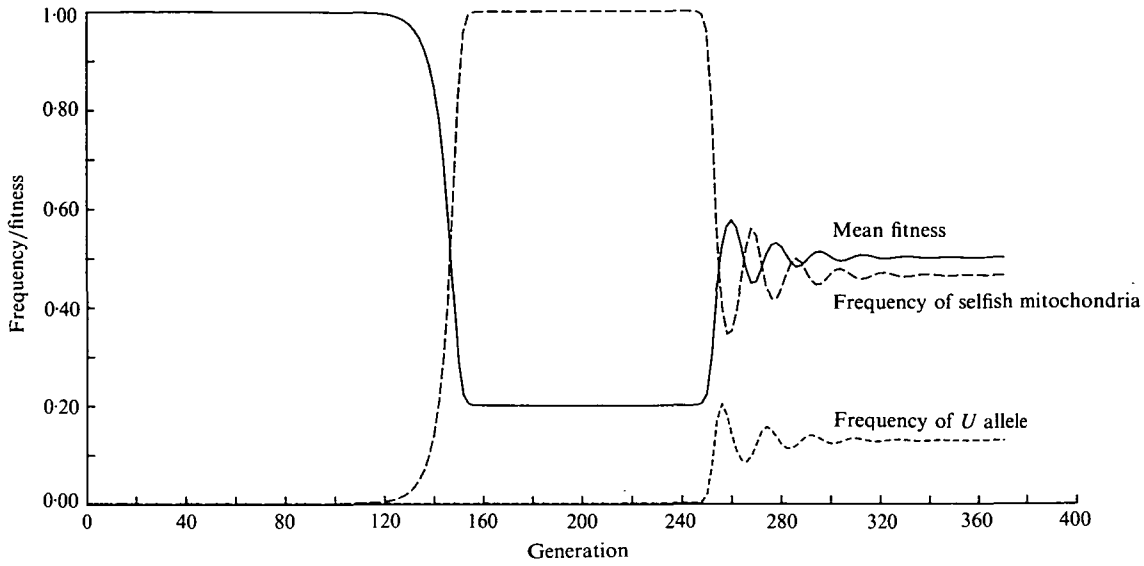

Fig. 3. Population dynamics of selfish mitochondria (introduced at generation 30) and the *U* allele (introduced at generation 200) under the following set of parameters: $r = 1{\cdot}2$, $a = 0{\cdot}2$, 5 mitochondria per gamete and 10 mitotic divisions between meiosis.

An example of the dynamics of the process is given on Fig. 3; after introduction of the *U* allele the system exhibits a damped oscillation around its eventual equilibrium level due to this time lag.

Two elaborations of the above model were investigated; they do not alter the general results and are included for completeness and to demonstrate that the conclusions are robust. First, it was assumed that gamete phenotype is determined by its haploid genotype. This may have been the case in the earliest evolution of sexual life-cycles as 'lower' organisms spend most of their life-cycle as haploids. However, in many extant species gamete morphology is determined by the diploid genotype. In the diploid case, if *U* is dominant then *B*-bearing gametes from *UB* individuals will also exclude cytoplasm during fertilization and will have fitness ω. This increases selection against the *B* allele and the frequency of *U* increases. If *U* was recessive it would not be able to spread as it could not become associated with a purely w.t. cytoplasm (it would be 'infected' with selfish mitochondria when *U*-bearing gametes from *UB* individuals fused with gametes containing selfish mitochondria). The second assumption was that zygotes formed from two *U*-bearing gametes have a 50% probability of inheriting either cytotype. If *U*/*U* fusions are lethal, the mean fitness of *U* alleles is $\omega(1\text{-}f_U)$, where f_U is the frequency of *U* alleles, resulting in frequency dependent selection. In the initial stages of invasion f_U is, by definition, low so *U* alleles can still invade a population but do not reach such high equilibrium frequencies as in the examples shown on Table 3. Moreover lethal *UU* fusions are likely to be a transitory phenomenon prior to the evolution of a mechanism preventing such fusions, as is observed in extant species. If *UU* fusions inherit cytoplasm from both gametes, the *U* allele is unable to invade, as individual alleles cannot become linked to a cytoplasm containing only w.t. mitochondria.

4. Discussion

Several other investigations have been made into the population genetics of mitochondrial genomes, generally to assess their use in elucidating population structure or tracing maternal lineages (reviewed in Birky, 1991). They were inappropriate for investigating the population genetics of deleterious cytoplasmic genomes as they assume neutrality (e.g. Takahata & Maruyama, 1981; Birky *et al.* 1983, 1989; Takahata & Palumbi, 1985; Chapman *et al.* 1982), or assume that mitosis results in gametes containing a single type of mitochondrion (e.g. Takahata, 1984; Takahata & Palumbi, 1985). The model closest to the one developed here is that of Takahata & Slatkin (1983) who assumed selection acted on the zygote and was determined by its intra-cellular genotype. They assumed no selection occurred during mitosis but simulated random drift by sampling during mitosis. The model developed here is similar but with the addition of selection and replicative differences during mitosis.

For any set of life-cycle parameters (number of mitochondria in cells and gametes, number of mitotic divisions between gametogenesis), there appears to be a combination of replicative advantage and metabolic activity which allows selfish mitochondria to invade (Table 1). Given the large number of mitochondria in a population it seems inevitable that such a mutation will eventually occur and it appears that the load (in terms of reduced fitness) imposed on a population may be substantial. The results presented in Table 2 suggest fitness values as low as 0·05 are plausible and that a population may be driven to near extinction by

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a selfish mitochondrion which completely lacks metabolic activity but which has a reproductive rate five times greater than the wild type. Moreover, mutations producing selfish mitochondria or more virulent strains of intracellular parasites are likely to arise continuously and their gradual, sequential accumulation in the population may result in a continuous decline in mean fitness.

Selfish mitochondria are likely to impose another, less direct, cost on populations. If the presence of selfish genomes results in uniparental inheritance of cytoplasm, this makes the subsequent evolution of mitochondria essentially asexual. As in asexual nuclear genomes this invokes the operation of Muller's ratchet (i.e. deleterious mutations accumulate) and also slows the sequential incorporation of advantageous mutations. In sexual species this may be a force favouring the transfer of genes from organelle to nuclear DNA where they may gain the advantages inherent in Mendelian segregation and recombination.

The reproductive advantage r of selfish mitochondria may apply to either the organelle or a single allele within the genome of the organelle. Mitochondria in several organisms appear to recombine (Birky & Skavaril, 1976) and biased gene conversion to a metabolically less active allele (equivalent to a higher rate of replication) will also result in the spread of deleterious mitochondria; an example of biased gene conversion in mitochondria is the ω locus in yeast (Dujon, 1981). The spread of such deleterious alleles may constitute another selection pressure favouring the transfer of genes from mitochondrial to nuclear DNA. Another pressure may be that mitochondria with smaller genomes replicate faster (Rand & Harrison, 1986).

There have been direct observations of deleterious cytoplasmic elements. They exist as intra-cellular parasites of ciliates (Beale & Knowles, 1978), are known to induce asexuality in several organisms (Hurst *et al.* 1990; Stouthamer *et al.* 1990) and the products of bacteria in sperm are known to kill zygotes if the egg did not also carry these bacteria (Roussett & Raymond, 1991); the latter example is a case where exclusion of cytoplasm is not complete presumably because bacterial products rather than complete genomes enter the zygote. Selfish mitochondria may also have been observed by Fisher & Skibinski (1990) in *Mytilus*. The example of selfish mitochondria is really an example of a sexually-transmitted disease (STD). The ultimate STD (transposable DNA) is known to exist, as are a range of conventional STDs known to infect animals. It seems inevitable that if cytoplasms are shared, an organism will evolve to exploit this opportunity for transmission. Thus both direct observation of deleterious cytoplasmic agents and indirect evidence from the widespread occurrence of STDs suggest a significant fitness reduction may result from sharing cytoplasms. Thus the first condition favouring an U allele appears to exist, i.e. the widespread existence of deleterious cytoplasmic agents.

The second condition, that a mutation to a primitive U allele may be physiologically plausible is supported by two lines of molecular evidence. Firstly, gamete recognition, binding and fusion are generally independent processes utilizing different proteins. Secondly, gametes may spontaneously take up DNA from their environment. Spontaneous uptake of exogenous DNA is known to occur in bacteria (Hoelzer & Michod, 1991 and references therein) and has been demonstrated in mammalian sperm by Lavitrano *et al.* (1989), (although claims that such DNA was subsequently transmitted to the offspring remain controversial), and mammalian cells will take up exogenous DNA under certain conditions (Wigler *et al.* 1979). Thus a simple mutation in the cell fusion system (which need not affect gamete recognition or binding) may bring gametic DNA in close enough proximity that spontaneous uptake will occur without the transfer of cytoplasm. Gene transfer will presumably be less efficient and incur a reduction in fitness: this can be incorporated into the model by further reducing the value ω.

As explained previously, the spread of U alleles can be attributed to their becoming 'linked' to a cytoplasm containing only w.t. mitochondria. In this model the cytotype in which the mutation to the U allele occurs it not important as sampling will eventually 'link' it to a purely w.t. cytoplasm. In cases where selfish mitochondria are virtually fixed in the population the probability of this linkage occurring, although finite, may be exceedingly small. Two lines of argument overcome this objection. Firstly, primitive sexual lifecycles may have used small gametes containing a small number of mitochondria together with a large number of mitotic divisions (possibly to inhibit the spread of cytoplasmic agents) which makes linkages more plausible. Second, it seems plausible that mutations to selfish mitochondria or mutations of existing intracellular parasites to more virulent forms may be reasonably common; any pre-existing U alleles maintained in the population by a mutation/selection balance could not become associated with such agents and would subsequently spread through the population.

In extant species it appears that a small amount of paternal 'leakage' may occur (e.g. Kondo *et al.* 1990; Gyllensten *et al.* 1991; references therein). This does not alter the general conclusions of this model as the small proportion of paternal mitochondria entering the zygote (between 0·05 and 10^{-4}) may be lost by sampling and selection during mitotis (e.g. Fig. 2*b*) although the rate of loss is sensitive to the parameters governing sampling, i.e. replication rate, activity and the number of mitotic divisions which makes the dynamics of paternal leakage complex. The examples on Table 3 show a four-fold decrease in the frequency of the U allele in the presence of a small (2 or 4%)

amount of paternal leakage (or a 40-fold decrease in the case of $a = 0{\cdot}2$ and 4% leakage). A significant amount of paternal leakage is likely to be a transitory phenomenon as natural selection will favour U alleles which reduce this leakage. Presumably the amount of leakage observed in extant species represents a balance between the benefit of decreased leakage which inhibits the spread of deleterious cytoplasmic agents and the advantages of increased leakage which aids the incorporation of advantageous mutations across lineages (e.g. Takahata, 1984).

There are two types of U alleles which result in uniparental inheritance of cytoplasm: one which excludes cytoplasm from the other gamete at fertilization (the type investigated here) or an allele which excludes cytoplasm from the gamete in which it is expressed; because of the similarity to the mammalian system these will be subsequently referred to as X and Y alleles respectively. Hoekstra (1987, 1990*a*) investigated the dynamics of a Y allele in the presence of deleterious cytoplasmic genomes but had to invoke implausible conditions to allow its spread. The model described here can be modified to investigate this type of allele and its inability to spread can be attributed to its lack of 'linkage' with its cytoplasm (in contrast to the X allele which becomes linked to an exclusively w.t. cytoplasm). However, a Y allele may spread under the following circumstances.

(i) If destructive conflict occurs between organelles derived from different parents (for example mutual destruction of mitochondria) resulting in reduced viability of the zygote. A system of uniparental inheritance of cytoplasm based on Y alleles avoids this conflict and could be stable but the conditions necessary to allow its evolution are less plausible than that of a X allele. Firstly, an organelle must arise, by mutation, which kills other mitochondria but which is itself not susceptible (an event likely to much less frequent than mutation to a 'selfish' form). Secondly, such a mutation would rapidly spread to fixation and conflict between organelles could only subsequently arise if organelles carrying this mutation could recognize their origin i.e. an interaction occurs with nuclear genes which 'marks' the organelles as being derived from one particular gamete. This may occur in *Chlamydomonas*, the slime mould *Physarum* and some conifers (Kuroiwa *et al.* 1982; Neale *et al.* 1989; Harrison & Doyle, 1990; Bennoun *et al.* 1991; Meland *et al.* 1991) although these examples do not demonstrate that conflict is deleterious, rather it appears to be the mechanism by which uniparental inheritance is achieved.

(ii) If two or more types of deleterious agents are segregating in the population and any mixing which occurs in biparental inheritance is inherently deleterious (Hurst, 1990). However a quantitative analysis of the dynamics of such a system was not attempted.

It should be noted that even if these circumstances did arise and Y alleles started to increase in frequency, then prior to the evolution of strictly uniparental inheritance of cytoplasm (mediated by restricting fertilization to Y- and *non*-Y-bearing gametes) the population would still be susceptible to invasion of selfish organelles which would favour X alleles. The X alleles share the same property of the Y alleles in determining uniparental inheritance of cytoplasm and, as shown above, can avoid infection with selfish mitochondria; the X allele would therefore spread at the expense of the Y. Thus, the most parsimonious evolutionary explanation for the evolution of uniparental inheritance seems to be a system initially based on a X allele which prevents cytoplasm from the other gamete entering the zygote at fertilization. As this model requires only a single nuclear mutation it is also more parsimonious than models which involve the prior evolution of mating types (e.g. Hoekstra, 1990*b*; Hurst & Hamilton, 1992; Law & Hutson, 1992).

The results presented above support the theory that uniparental inheritance of cytoplasm arose in response to the presence of deleterious cytoplasmic agents. Uniparental inheritance involves a fitness reduction to 0·5 (the two-fold cost of sex) and the results of Table 2 suggest the fitness of an isogamous sexual population will fall below 0·5; hence on a group selection argument uniparental inheritance may be expected to evolve. The analysis of the population genetics of the U allele, examples of which are shown in Table 3, suggest it may arise by individual selection.

Once a U allele is established in a population anisogamy may evolve. The cytoplasm of B-bearing gametes may be discarded at fertilization (in a U/B fusion) while that of U-bearing gametes is less likely to be discarded (but will depend on the frequency of U alleles and the assumptions concerning UU fusions). Hence it may be advantageous for an individual to produce smaller B-bearing gametes if this allows a greater number to be produced. This constitutes disruptive selection on gamete size, and genes closely linked to, or regulated by, the U/B locus may be selected to alter the size of gametes, depending on the genotype at this locus (the heritability of gamete size, at least in mammals appears high, $h^2 = 0{\cdot}6$–$0{\cdot}9$; Beatty, 1972). This disruptive selection on gamete size will rapidly lead to anisogamy (Parker *et al.* 1972; Maynard-Smith, 1978). Once significant anisogamy has evolved, selection will favour mechanisms preventing fusions between smaller gametes as the resultant zygotes may have insufficient cytoplasmic provisioning for survival (Parker *et al.* 1972). Fertilizations will then occur predominantly between U- and B-bearing gametes and their relative frequencies will stabilize at 0·5 (Fisher, 1930). Under this scenario the U and B alleles become equivalent to the familiar 'X' and 'Y' alleles (or chromosomes) which determine sex in higher organisms.

Uniparental inheritance does not invariably lead to disruptive selection on gamete size and anisogamy. In

some situations both types of gametes may be small, for example if the optimal reproductive strategy of both alleles is to produce a large number of very small gametes. Alternatively large 'male' gametes may be selected if this increases their chance of survival, for example to enable them to survive adverse conditions. Under these circumstances anisogamy may not arise, but the U and B alleles will still control uniparental inheritance of cytoplasm and may eventually give rise to 'mating types' as occur in many organisms such as fungi.

The standard theory of the evolution of sexual reproduction assumes it begins with isogamy (which has no overt cost), and that the 'twofold cost' arises later as uniparental inheritance of cytoplasm evolves. The changes in fitness incurred by a population evolving sexual reproduction in the presence of deleterious cytoplasmic agents are markedly different (Fig. 3). It appears that a rapid decrease in mean fitness may occur as isogamy allows the accumulation on deleterious cytoplasmic agents and mean fitness can only subsequently rise to a maximum of ω as uniparental inheritance of cytoplasm evolves. As mechanisms arise which restrict fertilizations to type U/B the size of U-bearing gametes will become optimal, and mean fitness will rise to a 0·5 (the twofold cost of sex) relative to the asexual or isogamous alternatives. This suggests that a significant fitness barrier may be associated with the transition from asexual to sexual reproduction.

This analysis therefore suggests that deleterious cytoplasmic genomes may have three main effects on the evolution of sexual reproduction. Firstly, they constitute a fitness barrier to the transition from asexual to isogamous sexual reproduction. Secondly, they explain the near-universal mechanism by which it is achieved, that is by uniparental inheritance of cytoplasm. Thirdly, they are indirectly responsible for the 'twofold cost of sex' and hence whether it is evolutionarily stable compared to the alternative of asexuality.

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Appendix

The twofold cost of sex

As many geneticists seem unaware that the 'twofold cost of sex' is due to the uniparental inheritance of cytoplasm rather than an inherent cost of sexual reproduction, I include the following explanation. For simplicity, the term anisogamy is used to describe a population with uniparental inheritance of cytoplasm, as this enables use of the familiar terms 'male', 'female', 'egg' and 'sperm'.

Assume each individual has 10 Ue (where Ue is an arbitrary unit of energy) available for reproduction, and the optimal size of a zygote is 2 Ue. Each asexual individual can produce five zygotes of 2 Ue, each of which contains its diploid genome i.e. $2n$, hence its contribution to the next generation is $5 \times 2n = 10n$. A sexual individual in an isogamous population may produce 10 gametes of 1 Ue, each of which is haploid. Assuming all are fertilized and zygotes received cytoplasm from both gametes (giving zygotes of 2 Ue), an individual's contribution to the next generation is $10n$, the same as for asexual individuals. A female in an anisogamous population produces five eggs of size 2 Ue (since the sperm are assumed to make a negligible energetic input to the zygote) each of which is haploid. A females contribution to the following generation is $5n$, half that of an individual in an asexual of isogamous population – this is the 'twofold cost of sex'. The above argument is based on individual selection whereas a population (in reality group-selection) argument is sometimes invoked. Assuming each population has 10 individuals, the number of offspring produced by an asexual population is $10 \times 5 = 50$ zygotes, and the number produced by an isogamous sexual population is $10 \times 10 = 100$ gametes = 50 zygotes. An anisogamous population will contain five females and five males, hence the number of zygotes produced is $5 \times 5 = 25$, half the number produced by asexual or isogamous populations. There are other, largely unquantified, costs of sex such as the effort, risk, and uncertainty of finding partners, but these do not constitute the 'twofold' cost.

The above result relies on the assumption that male gametes make no energetic contribution (in the form of cytoplasm) to the zygote. Provided its cytoplasm is excluded from the zygote at fertilization, the size of the male gamete is immaterial. This demonstrates that the 'twofold cost of sex' is due to the uniparental inheritance of cytoplasm, anisogamy being a common mechanism by which this is achieved. In many anisogamous organisms significant energetic input in the form of paternal care is present; for example the extent of paternal care observed in many birds and mammals is such that it swamps any energetic differences between the gametes. The results presented in the main text explain why significant paternal contribution in terms of paternal care may evolve whereas paternal contribution in terms of increased cytoplasmic contribution is absent in higher organisms (such a contribution would effectively become a 'poison chalice' of deleterious cytoplasmic genomes).

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