1 **EVOLUTION OF MATING TYPES DRIVEN BY PURIFYING SELECTION AGAINST**

2 **MITOCHONDRIAL MUTATIONS**

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Abstract

9 Sexual cell fusion combines genetic material of two gametes, but why the two reproductive cells
10 have to belong to distinct self-incompatible gamete classes is not known. In a vast majority of
11 sexual eukaryotes, mitochondria are inherited uniparentally from only one of the two mating types,
12 which is thought to facilitate purifying selection against deleterious mitochondrial mutations and
13 limit the inter-genomic conflicts. Here I argue that two mating types in eukaryotes represent a
14 mechanism of mitochondrial quality control through the highly asymmetric transmission of
15 mitochondrial genes at cell fusion. I develop a mathematical model to explicitly study the evolution
16 of two self-incompatible mating type alleles linked to the nuclear locus controlling the pattern of
17 organelle inheritance. The invasion of mating-type alleles is opposed by the short-term fitness
18 benefit of mitochondrial mixing under negative epistasis and the lower chance of encountering a
19 compatible mating partner. Nevertheless, under high mitochondrial mutation rates purifying
20 selection against defective mitochondria can drive two mating types with uniparental inheritance
21 to fixation. The invasion is further facilitated by the paternal leakage of mitochondria under
22 paternal control of cytoplasmic inheritance. In contrast to previous studies, the model does not rely
23 on the presence of selfish cytoplasmic elements, providing a more universal solution to the long-
24 standing evolutionary puzzle of two sexes.

25 **Keywords:** mating types, mitochondria, uniparental inheritance, purifying selection, sexes

26

Introduction

28 Sex is among the traits universal to all complex life and was therefore already present in the last
29 eukaryotic common ancestor. By combining the genetic material of two gametes, sexual cell fusion
30 and meiotic recombination exposes the hidden genetic variation in finite populations, breaks up
31 the unfavorable allelic combinations under fluctuating selection, and mitigates the mutational
32 meltdown (Otto, 2009). In a vast majority of cases, the two mating partners belong to distinct self-
33 incompatible gamete classes, i.e. mating types in isogamous protists or true sexes with
34 anisogametes, but the selective forces behind this fundamental asymmetry remain elusive (Billiard

1 et al., 2010). The existence of two gamete types in most eukaryotes is often regarded as an
2 evolutionary conundrum, as it reduces the number of potential mating partners, which should be
3 detrimental if the cost of finding a compatible gamete is high and the mating opportunities are
4 limited.

5 Several non-exclusive explanations for the emergence of self-incompatible gamete types in a
6 unisexual population have been proposed over the years (Billiard et al., 2010). Two mating types
7 might have appeared together with bipolar gamete-recognition systems ensuring efficient inter-
8 cellular signaling (Hoekstra 1982; Hadjivasiliou et al., 2015), to promote outbreeding
9 (Charlesworth and Charlesworth, 1979, Uyenoyama, 1988) or to improve the mitochondrial-
10 nuclear coadaptation (Hadjivasiliou et al., 2012). Particularly appealing has been the idea that
11 mating types emerged to ensure the asymmetric inheritance of cytoplasmic genetic elements.
12 Indeed, in a vast majority of eukaryotes, only one gamete class transmits its organelles—
13 mitochondria and chloroplasts—to the zygote, although the uniparental inheritance is not always
14 complete, with paternal leakage and heteroplasmy being relatively common (Breton and Stewart,
15 2015). Early theoretical studies supported the hypothesis, but relied on the presence of the so-
16 called "selfish" or parasitic cytoplasmic elements, simplistic assumptions, and lacked generality
17 or empirical support (Hastings, 1992; Hurst and Hamilton, 1992; Hutson and Law, 1993).

18 A more general view of the evolutionary advantage of uniparental inheritance (UPI) is that it
19 improves the efficacy of purifying selection against mitochondrial mutations (Bergstrom and
20 Pritchard, 1998; Hadjivasiliou, 2013), and therefore confers a long-term fitness advantage.
21 Mitochondrial mixing, on the other hand, reduces the strength of selection and is costly in a long
22 term (Radzvilavicius, 2016). Asymmetric transmission of mitochondria therefore counters the
23 mutational meltdown in mitochondrial genomes, at least partially accounting for the highly
24 efficient purifying selection in the absence of recombination (Cooper et al., 2015). A recent study
25 explicitly accounting for the segregation of detrimental mitochondrial mutations, however, found
26 that the fitness benefit of UPI decreases in a frequency-dependent manner, and concluded that UPI
27 alone is unlikely to drive the evolution of two self-incompatible mating types in an ancestral
28 unisexual population (Hadjivasiliou, 2013). The robustness and generality of these results,
29 however, is limited by the assumptions of the study; in particular, the peculiar mating kinetics in
30 which gametes unable to find a suitable mating partner immediately incur a severe fitness cost are
31 not representative of biological reality, and put the self-incompatible gametes at a strong
32 disadvantage. Whether the asymmetric inheritance of mitochondria can facilitate the evolution of
33 binary mating types under the mitochondrial mutation pressure remains unclear.

34 Here I develop a mathematical model to investigate whether selection against deleterious
35 mitochondrial mutations can drive the evolution of two self-incompatible mating types by
36 establishing the asymmetric transmission of mitochondrial genes. Mitochondria continuously
37 accumulate mutations which are purged by selection on the level of the cell, with random
38 mitochondrial drift generating additional variance at every cell division. Nuclear inheritance-
39 restriction alleles regulate the selective destruction of mitochondria inherited either from the cell

2

1   carrying the allele, or its mating partner in the opposite nuclear state. Mating kinetics play a central
2   role in the model, with long mating periods and low gamete mortality rates favoring the invasion
3   of self-incompatible gamete classes. Strikingly, paternal leakage of mitochondria—an
4   evolutionarily stable state under paternal control of cytoplasmic inheritance—can facilitate the
5   evolution of binary mating types under negative epistasis between mitochondrial mutations.

6

Modelling the evolution of uniparental inheritance

8   **Two modes of UPI regulation.** Mechanisms of uniparental inheritance vary substantially across
9   eukaryotic species (Sato and Sato, 2013), but one general theme seems to be rather common: one
10 of the mating partners tags its mitochondria with the mating-type specific marker protein, which
11 is recognized by the partner's molecular factors after the gamete union, leading to the eventual
12 degradation of the marked organelles. In mammals, for example, sperm mitochondria are tagged
13 by the recycling marker protein ubiquitin and destroyed by the egg's cytoplasmic destruction
14 machinery after the gamete union (Sutovsky et al., 1999). Similarly, in basidiomycete yeast
15 *Cryptococcus neoformans,* genes *SXI1a* and *SXI2α* located in opposite mating types are
16 responsible for tagging and recognition of paternal mitochondria (Yan et al., 2007).

17 In the present work I assume that uniparental inheritance can be established in two ways, based on
18 how the two gametes control organelle transmission (Fig. 1). First, a cell (wild-type allele *B*) can
19 develop the ability to recognize a universal mitochondrial marker protein, at the same time
20 protecting its own organelles from degradation, e.g. by ceasing the expression of the marker in its
21 own mitochondria (allele U_m, Fig. 1b). I term this mechanism the "maternal" mode of UPI, and
22 the gamete destroying its partner's mtDNA the "maternal" gamete—the definition which does not
23 rely on the prior existence of mating types or sexes and merely reflects the fact that the gamete
24 controlling the cytoplasmic inheritance eliminates its partner's mtDNA, but not its own (Fig. 1b).
25 Alternatively, a cell can start producing a new nuclear-coded and universally recognized
26 mitochondrial marker, but lose the ability to recognize the tag in its own cytoplasm (allele U_p). In
27 this case the gamete essentially controls the inheritance of its own organelles, as stopping the
28 expression of the mitochondrial tag protein would make them unrecognizable in the zygote. I term
29 this mechanism the "paternal" mode of UPI (Fig. 1c). In gamete unions with identical alleles at
30 the mitochondrial-inheritance locus, both mating partners lack either the mitochondrial marker or
31 the corresponding molecular destruction machinery. The inheritance of mitochondria is therefore
32 biparental in $U_m \times U_m$ and $U_p \times U_p$ gamete unions.

33 **Mating kinetics.** The rules governing the frequency of gamete unions of distinct mating types are
34 expected to play a critical role in the origin and evolutionary stability of mating types (Iwasa and
35 Sasaki, 1987). Rare sexes, for instance, are favored in models where mating opportunities are
36 limited and only a short period of time is available to locate a suitable mating partner, but the same
37 models penalize the newly emerging self-incompatible gamete classes in unisexual populations
38 where all gametes are initially compatible. A more realistic scenario should assume that the mating

1 period can be significantly longer than the duration of a single cell-fusion attempt, and so the
2 gametes can wait for a suitable mating partner to arrive before being eliminated from the
3 population.

4 In this work I adopt the mating kinetics first developed in the models of Iwasa and Sasaki (1987),
5 and Hutson and Law (1993). I assume the gamete pool of finite size, in which the influx of gametes
6 matches their removal due to random death and zygote formation. The gamete influx rates are
7 proportional to the allele frequencies within the infinite population, while the gamete death rate is
8 kept at a constant level δ. Cells within the gamete pool form random pairwise associations at rate
9 $\beta/2$, and are removed from the pool if they are compatible and able to fuse. The allele frequencies
10 in the next generation are calculated from the steady-state gamete-class frequencies within the
11 mating pool. These frequencies correspond to the equilibria of the following system of equations

12
$$\frac{\mathrm{d}F_i}{\mathrm{d}t} = \begin{cases} -\delta F_i - \beta F_i + \delta f_i + \beta f_i \left(1 - \sum_{k \in \text{s.i.}} F_k^2\right), & \text{if } i \text{ is universally compatible} \\ -\delta F_i - \beta F_i(1 - F_i) + \delta f_i + \beta f_i \left(1 - \sum_{k \in \text{s.i.}} F_k^2\right), & \text{if } i \text{ is self incompatible} \end{cases}$$

13 Here F_i denotes the frequency of the genotype i in the gamete pool, while f_i is the corresponding
14 frequency within the infinite population. The sums here are over the self-incompatible gamete
15 classes (s.i.). I fix the mating rate at $\beta = 1$ and vary only the value of δ, as the steady-state
16 frequencies of gamete classes depend only on the ratio of these rates β/δ.

17 **Population life cycle.** The model assumes an infinite population of unicellular haploid organisms,
18 each containing M mitochondria, and is in many ways similar to the model developed in
19 (Radzvilavicius, 2016). The population state can be represented by the $(M+1) \times n$ matrix $\mathbf{P}$,
20 where the matrix element $P_{i,j}$ denotes the frequency of cells in a nuclear state $j \leq n$ and containing
21 m mutant mitochondria. The horizontal index j enumerates all possible nuclear states including
22 the mating type and the mode of mitochondrial transmission.

23 Mitochondrial mutation is represented by the transition $\mathbf{P}^{(t,1)} = \mathbf{U}\mathbf{P}^{(t)}$, where the matrix element
24 $U_{i,j}$ represents the probability that a cell with j mutant mitochondria will have i mutants after the
25 mutation event,

29
$$U_{i,j} = \binom{M-j}{i-j} \mu^{i-j}(1-\mu)^{M-i}; \; i,j \in [0, M].$$

26 Mutation occurs in individual mitochondria, but selection acts on the level of the cell, i.e. on
27 groups of mitochondria. In our model, cell fitness w directly depends only on the mitochondrial
28 part of cell's genotype. The updated population state after selection is then

30
$$\mathbf{P}^{(t,2)} = \frac{(\mathbf{Iw})\mathbf{P}^{(t,1)}}{\mathbf{w}^{\mathrm{T}}\mathbf{P}^{(t,1)}\mathbf{u}_n},$$

4

1 where $\mathbf{I}$ is the identity matrix. $\mathbf{u}_n$ is a column vector of ones, so that $\mathbf{P}^{(t,1)}\mathbf{u}_n$ is the row-wise sum

2 of $\mathbf{P}^{(t,1)}$ and $\mathbf{w}$ is a column vector containing all possible values of mitochondrial fitness,

8
$$w_i = 1 - \left(\frac{i}{M}\right)^{\xi} \; ; \; i \in [0, M].$$

3 Parameter ξ determines the magnitude of epistatic interactions between mitochondrial mutations.

4 With $\xi > 1$ the fitness cost of every new mutation increases with the overall mutation load

5 (negative epistasis). Negative epistasis between deleterious mitochondrial mutations leads to

6 mitochondrial threshold effects, well known from empirical observations in eukaryotes

7 (Rossignol et al., 2003).

9 Gametes in the gamete pool fuse at random, according to their equilibrium frequencies F_i. For

10 asymmetric gamete unions, assuming that the gamete k is of maternal type, we have,

11
$$\mathbf{z}_{kl} \sim \begin{cases} (\boldsymbol{\Phi}^{(\pi)}\mathbf{P}_{\bullet,k}^{(t,2)}) * (\boldsymbol{\Psi}^{(\pi)}\mathbf{P}_{\bullet,l}^{(t,2)}) \dfrac{2^{\delta_{k \neq l}} F_k F_l}{f_k f_l}, & \text{if } k \text{ and } l \text{ are compatible} \\ \mathbf{0}, & \text{otherwise} \end{cases}$$

12 An asterisk here denotes vector convolution, f_k is the frequency of cells in a nuclear state k, i.e.

13 $f_k = \mathbf{u}_{M+1}^T \mathbf{P}_{\bullet,k}^{(t,2)}$ and F_k is the corresponding equilibrium frequency in the gamete pool. The delta

14 symbol $\delta_{k \neq l} = 1$ if $k \neq l$ and is 0 otherwise. The zygote-state vectors $\mathbf{z}_{kl}$ are scaled linearly to

15 sum up to one. The two transition matrices $\boldsymbol{\Phi}^{(\pi)}$ and $\boldsymbol{\Psi}^{(\pi)}$ are included to implement the

16 mitochondrial inheritance bias where one of the gametes transmits more mitochondria than the

17 other. We assume that the paternal gamete contributes πM mitochondria through sampling without

18 replacement, and that $(2 - \pi)M$ mitochondria come from the maternal gamete via random

19 sampling with replacement ($\pi = 0, \frac{1}{M}, \dots, \frac{1-M}{M}, 1$). The two transition matrices therefore have

20 elements

22
$$\Phi_{i,j}^{(\pi)} = \binom{[2 - \pi]M}{i} \left(\frac{j}{M}\right)^i \left(1 - \frac{j}{M}\right)^{(2-\pi)M-i} \; ; \; i \in [0, (2 - \pi)M], \; j \in [0, M],$$

21 and

23
$$\Psi_{i,j}^{(\pi)} = \binom{j}{i} \binom{M - j}{\pi M - i} \binom{M}{\pi M}^{-1} \; ; \; i \in [0, \pi M], j \in [0, M].$$

24 The life cycle is completed by two meiotic cell divisions restoring the haploid state. At the start of

25 the next generation the genotype frequencies then are

26
$$\mathbf{P}_{\bullet,k}^{(t+1,0)} = \mathbf{F}_2 \mathbf{F}_1 \left(\mathbf{z}_{kk} + \frac{1}{2}\sum_{l \neq k} \mathbf{z}_{kl}\right).$$

27 I do not differentiate between $\mathbf{z}_{kl}$ and $\mathbf{z}_{lk}$, i.e. both state vectors indicate the same zygote type. $\mathbf{F}_1$

28 and $\mathbf{F}_2$ are transition matrices for the two meiotic divisions implemented as mitochondrial

29 sampling without replacement. Their corresponding elements are

2

$$F_{(1)i,j} = \binom{2j}{i} \binom{4M - 2j}{2M - i} \binom{4M}{2M}^{-1} ; i,j \in [0,2M],$$

1 and

3

$$F_{(2)i,j} = \binom{j}{i} \binom{2M - j}{M - i} \binom{2M}{M}^{-1} ; i \in [0, M], j \in [0,2M].$$

4 The following results are based on the numerical solution of the above system of equations.

5

6 Asymmetric sex before mating types: UPI facilitates purifying selection against deleterious
7 mitochondrial mutations

8 Eukaryotic sex depends on molecular mechanisms for self/non-self-recognition and gamete
9 attraction (Goodenough and Heitman, 2014); similar mechanisms likely existed in both the
10 bacterial ancestor of mitochondria and the archaeal host in the form of quorum-sensing and
11 biofilm-formation systems triggered by external conditions (Ng and Bassler, 2009; Fröls, 2013).
12 With the prokaryotic ancestry of eukaryotic gamete attraction machineries, the initial proto-
13 eukaryotic self-recognition system was likely symmetrical, i.e. initial forms of sexual reproduction
14 did not involve differentiation into distinct gamete classes (Goodenough and Heitman, 2014;
15 Heitman, 2015). Self-incompatible mating types and sexes then could have been a later addition,
16 driven, as I shall argue here, by purifying selection against mitochondrial mutations.

17 Assuming that the initial form of sexual reproduction was unisexual, I first model the evolution of
18 uniparental inheritance in an ancestral population without mating types. While all pairs of gametes
19 are capable of mating, only the unions between U_m and B (or U_p and B) involve the asymmetric
20 transmission of mitochondria (Fig. 1). As I show in Fig. 2, both nuclear alleles U_m and U_p invade
21 and can reach the frequency of $e_2 = 0.5$, but the invasion dynamics differ substantially between
22 the two modes of UPI control.

23 Starting at low allele frequencies, the invader U_m attains one of two distinct equilibria, $e_1 < 0.5$
24 under low mutation rates and strong negative epistasis, or $e_2 = 0.5$ under higher mutation rates
25 (Fig. 2a). The analogous set of equilibria exists for the paternal invader U_p, but this time $e_2 = 0.5$
26 is the only asymmetric equilibrium which can be reached starting from low initial mutant
27 frequencies (Fig. 2a). In this case the combination of the mutation rate and the initial frequency
28 must exceed the unstable equilibrium $0 < e_1' < 0.5$, the characteristic frequency of which
29 approaches zero at high mutation rates. Paternal leakage of mitochondria relaxes the conditions
30 for the invasion of U_p to the equilibrium e_2 (Fig. 2b), but at the same time hinders the invasion of
31 U_m under low mutation rates.

32 What determines the equilibrium frequencies of UPI alleles in a population without mating types?
33 The model shows that the fitness advantage of U_m decreases as the allele invades from small
34 frequencies (Fig. 2c), while the opposite is true for U_p (Fig. 2d), determining the locations of

6

1   equilibria e_1 and e_2. This behavior can be explained in terms of costs and benefits of asymmetric
2   transmission of mitochondrial genes, and the statistical association of these benefits to the nuclear
3   allele of mitochondrial inheritance control.

4   Uniparental inheritance of mitochondria increases the frequency of extreme cytotypes
5   underrepresented at the mutation-selection equilibrium, reducing the abundance of the common
6   intermediate cytoplasmic states (Fig. 2e). This increase in mitochondrial variance facilitates
7   purifying selection against deleterious mutations and gives the UPI mutant a long-term advantage
8   over the biparental population. Nevertheless, under negative epistasis (Fig. 2e) intermediate
9   cytoplasmic states have higher fitness than expected from the linear combination of the extremes,
10   penalizing the UPI invader and giving the biparental inheritance a short-term benefit (Fig. 2f).
11   These short-term fitness effects, however, are relevant only if the association between the
12   cytoplasmic state and the nuclear allele of inheritance control is weak (e.g. with paternal leakage,
13   when part of the mitochondrial population is inherited from the unrelated gamete); otherwise the
14   long-term variance-based effects dominate. The stable equilibria e_1 and e_2 are located where short-
15   term effects match the long-term fitness advantage of the UPI invader.

16   Since the rate at which the resident B inherits the cytoplasm from the uniparental mutant rises with
17   increasing frequency of U_m, the short-term fitness gains of B increase, halting the invasion at $e_1 <$
18   0.5 or $e_2 = 0.5$. Paternal leakage associated with U_m weakens the mito-nuclear associations and
19   therefore reduces the strength of the long-term fitness effects. The opposite pattern of nuclear-
20   cytoplasmic linkage applies to the invader with paternally-determined UPI: destroying part of
21   one's own mitochondria increases the level of transmission asymmetry, but weakens the statistical
22   associations between the mitochondrial population and the allele U_p. As the allele U_p invades from
23   small frequencies, the rate of biparental unions $U_p \times U_p$ increases, but so does the overall frequency
24   of uniparental transmissions. The short-term fitness gains of invading U_p therefore increase with
25   its overall frequency. As the invasion proceeds, the biparental unions between the identical
26   gametes become more common, eventually reversing the trend of frequency-dependence (Fig. 2c-
27   d).

28

Evolving paternal leakage in UPI

30   Suppose now that the level of paternal leakage π is itself an evolvable trait, controlled by a single
31   nuclear allele U_p or U_m. The amount of organelles transmitted to the progeny from each gamete
32   can indeed be regulated genetically, e.g. by controlling the expression of gamete-specific protein
33   markers tagging the organelles for selective degradation (e.g. ubiquitin). What pattern of organelle
34   inheritance would we expect to evolve in the population without mating types? I performed
35   evolutionary invasion analysis to find the evolutionarily stable states (ESS; Eshel, 1983; Geritz et
36   al., 1998) for the paternal leakage π under both maternal and paternal modes of inheritance control.

With maternal regulation of cytoplasmic inheritance, the sole non-invadable state with asymmetric transmission of mitochondria is the strict UPI, i.e. $\pi_{\text{ESS}} = 0$ (Fig. 3a), at which the gamete U_m discards all mitochondria inherited from B. As the leakage π goes down, both the long-term fitness advantage of variance, and the strength of nuclear-cytoplasmic associations become more significant. The equilibrium frequency of U_m at π_{ESS} therefore increases with increasing mutant load, consistent with the role of UPI increasing the efficacy of purifying selection against mitochondrial mutations. Similarly, weak epistatic interactions ($\xi \to 1$) reduce the short-term fitness gains in symmetric gamete unions, and result in higher equilibrium frequencies of U_m (Fig 3b).

Under the paternal control of mitochondrial inheritance, the globally-attracting ESS lies between $0 \le \pi_{\text{ESS}} \le 1$ (Fig. 3c), with the frequency of U_p allele always at $e_2 = 0.5$. The deviation of π to either side of its π_{ESS} is detrimental: with $\pi < \pi_{\text{ESS}}$ the asymmetry of mitochondrial inheritance increases, but the strength of long-term mitochondrial-nuclear associations is diminished, benefiting the resident population; similarly, $\pi > \pi_{\text{ESS}}$ increases the strength of genetic linkage, but reduces the long-term effects of partial UPI—again to the detriment of the invader. The analysis further shows that the value of π_{ESS} goes down with increasing mutation rate μ and decreasing strength of epistasis ξ (Fig. 3d). The model therefore shows that limited mitochondrial mixing can be maintained in spite of its long-term fitness costs, but only if the inheritance-restriction allele causes the selective destruction of organelles inherited from the same mating type (paternal gamete by definition), and if the epistasis is negative.

Invasion of self-incompatible mating-type alleles establishes the population-wide UPI

The above analysis shows that in a population without mating types the following equilibria with asymmetric inheritance of mitochondria are possible, depending on mutation rates and the mode of UPI nuclear regulation:

1) U_m at $e_1 < 0.5$ with strict UPI ($\pi = 0$);
2) U_m at $e_2 = 0.5$ with strict UPI ($\pi = 0$);
3) U_p at $e_2 = 0.5$ with paternal leakage ($0 \le \pi \le 1$).

The last stable equilibrium for the allele U_p at $e_1 > 0.5$ (Fig. 2a-b) is not considered here, as it cannot be reached from low allele frequencies. These equilibrium frequencies do not depend on the gamete mortality rate δ, as all gametes are compatible in the absence of mating types. While the frequency of uniparental mutants U_m or U_p can reach 0.5, the overall rate of asymmetric unions cannot exceed 0.5 as long as matings between identical gametes ($U_m \times U_m$, $U_p \times U_p$, $B \times B$) are allowed. The biparental gamete unions can be eliminated, if a mutation in the intracellular signaling system leads to the incompatibility of cells carrying the same allele at the mitochondrial inheritance locus.

1   I next consider the invasion of self-incompatibility alleles Mt_1 and Mt_2, linked to either U_m/U_p or
2   B. Due to the inherent symmetry of the system, the invasion of U_mMt_1 under maternal inheritance
3   regulation is in fact equivalent to invasion BMt_2 under paternal control; similarly, the invasion of
4   U_pMt_1 is equivalent to the invasion of BMt_2 under maternal regulation of cytoplasmic inheritance
5   (Fig. 2a-b). It is therefore sufficient to study the evolution under maternal control, as the case of
6   paternal regulation can be obtained by simply reversing the order of allele invasion, given that the
7   initial equilibrium state exists and is stable under both modes of inheritance restriction.

8   With maternal allele U_m at equilibrium, the self-incompatible gamete class U_mMt_1 invades
9   replacing U_m, but generally to a lower frequency than the ancestral U_m (Fig. 4a-b). Linkage to the
10 mating type allele Mt_1 ensures that symmetric unions between gametes carrying U_m become
11 forbidden, increasing the frequency of uniparental matings and therefore leading to the higher
12 long-term fitness advantage of U_m associated with more efficient removal of deleterious mutations.
13 In a population consisting solely of U_mMt_1 and B, all gamete unions involving U_mMt_1 are therefore
14 uniparental. At the same time, however, the subpopulation of B enjoys higher short-term fitness
15 benefits of mitochondrial mixing in symmetric $B{\times}B$ unions, limiting the spread of the first mating
16 type allele.

17 Similarly, in the invasion of the second self-incompatibility allele Mt_2 linked to B, the gamete class
18 BMt_2 spreads at the expense of B, as it reduces the frequency of fully symmetric gamete unions
19 and ensures that BMt_2 inherits fit mitochondria from U_mMt_1 uniparentally more often than the wild-
20 type B. With the constant frequency of U_mMt_1, the fitness advantage of BMt_2 over B increases with
21 its frequency (Fig. 4c). The spread of BMt_2 also increases the long-term fitness advantage of the
22 first invader U_mMt_1 (Fig. 4d), as it reduces the short-term advantage of B. The spread of a second
23 mating-type allele therefore reinforces the fitness advantage of the first, allowing both to reach
24 high frequencies and even fix at low gamete mortality rates δ.

25

Conditions favoring fixation of mating types

27 The two mating type alleles fix only if they are capable of replacing the universally compatible
28 U_m/U_p and B, which is opposed by two forces: 1) the short-term fitness advantage of mitochondrial
29 mixing in subpopulations without mating types, and 2) the mating rate disadvantage of gametes
30 that can mate only with a subset of the population. Indeed, the spread and fixation of Mt_1 and Mt_2
31 is favored by the factors increasing the long-term fitness effects of asymmetric transmission of
32 mitochondria, and opposed by high gamete death rates δ.

33 First, regardless of the UPI regulation mode, the self-incompatible mating types replace the
34 unisexual residents under weak epistatic interactions between mitochondrial mutations ($\xi \to 1$,
35 Fig. 5). The short-term fitness advantage of mitochondrial mixing in biparental gamete unions only
36 applies to the case of negative epistasis between deleterious mutations (Radzvilavicius, 2016); at
37 $\xi = 1$, for example, the uniparental invaders become universally advantageous, with their

9

1   equilibrium frequency being limited only by the number of compatible mating partners. As the
2   strength of negative epistatic interactions increases ($\xi > 1$), the short-term fitness effects of
3   mitochondrial mixing become increasingly more important, opposing the spread of the two
4   mating-type alleles. This effect can be partially alleviated in the presence of paternal leakage, as it
5   tends to reduce the short-term fitness advantage of the universally-compatible gametes, favoring
6   the spread of the two mating types (Fig. 5). The results therefore indicate the importance of paternal
7   regulation of mitochondrial inheritance, as intermediate paternal leakage can be evolutionarily
8   stable only under paternal control of cytoplasmic inheritance. Under strong epistasis and high
9   levels of paternal leakage π, however, the first mating-type U_mMt_1 fails to replace the universal
10   U_m, which subsequently prevents the invasion of Mt_2 (Fig. 5).

11   High mitochondrial mutation rates favor the spread of mating-type alleles linked to the
12   mitochondrial-inheritance locus (Fig. 6). This is consistent with the long-term effect of asymmetric
13   mitochondrial transmission enhancing the efficacy of purifying selection against mitochondrial
14   mutations due to higher variance in mitochondrial mutation load (Fig. 2). As expected, the
15   evolutionary success of self-incompatibility alleles depends on the cost associated with the lower
16   frequency of potential mating partners within the population, which is represented by the gamete
17   mortality rate δ (Fig. 6). Under high mortality rates, gametes unable to find suitable mates are
18   rapidly eliminated from the gamete pool; as a consequence, the universal gametes carrying B or
19   U_m/U_p prevail. With low gamete death rates ($\delta \to 0$), on the other hand, the self-incompatible
20   mating types can persist longer, waiting for an encounter with a compatible mating partner, which
21   favors their invasion and eventual fixation (Fig. 6). Indeed, while gamete self-incompatibility
22   reduces the overall amount of potential mating partners within the population, with the evolution
23   of mating types and mating-type associated pheromone-based intracellular attraction systems,
24   gamete encounters are rarely random (Hadjivasiliou, 2015), and the part of the life cycle available
25   for mate-finding could indeed be much longer than the time separating random encounters between
26   mature reproductive cells.

27

Discussion

29   The existence of two sexes or mating types is often regarded as an evolutionary conundrum, as
30   both males and females are able to mate only with one-half of the population. The fitness benefits
31   of gamete differentiation must therefore exceed the costs associated with the reduced mating
32   opportunities. With the evolution of two mating types and the associated asymmetric signaling
33   systems (Hoekstra, 1982; Hadjivasiliou et al., 2015), the encounters between gametes are not
34   random, and the cost of being able to mate with only a part of the population might not be as high
35   as assumed in the previous studies (Hadjivasiliou, 2013). I showed, that two mating-type alleles
36   linked to the mitochondrial-inheritance locus can spread to fixation driven by the long-term
37   advantage of asymmetric transmission of mitochondrial genes, which increases the efficacy of
38   purifying selection against detrimental mitochondrial mutations, even though the invasion is

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1   opposed by the short-term fitness benefits of mitochondrial mixing in the biparental part of the
2   population, and the limited availability of potential mating partners. Two mating types should
3   therefore be expected to arise with fast accumulation of mitochondrial mutations, and high gamete-
4   survival rates ensuring that the fitness cost associated with the need to locate a suitable mating
5   partner is low. Additionally, the results show that paternal leakage of mitochondria can favor the
6   evolution of mating types under negative epistasis, suggesting that paternal control of cytoplasmic
7   inheritance could have been important.

8   Can the model account for the evolution of more than two gamete classes? Multiple mating types
9   with uniparental inheritance of mitochondria are indeed known. For example, in true slime mold
10 *Physarum polycephalum* with several mating types, uniparental inheritance of mitochondria via
11 selective organelle digestion follows a complex hierarchy according to the allele at one of the
12 mating-type loci (Moriyama and Kawano, 2003). Consider the invasion of a third self-
13 incompatible mating type Mt_3, linked to the mitochondrial inheritance locus with one of the
14 existing alleles, say U_m. The invader can therefore mate with both initial gamete classes, but part
15 of the gamete unions will remain biparental ($U_mMt_3 \times U_mMt_1$). The model shows that U_mMt_3 can
16 invade only if it has a large initial mating-rate advantage, i.e. under gamete mortality rates δ high
17 enough to compensate for the long-term fitness disadvantage due to less frequent uniparental
18 unions. Interestingly, unions between certain pairs of gamete types in *Physarum polycephalum* are
19 indeed biparental, indicating that some of the mating-type alleles might have arisen simply because
20 of their mating-rate advantage (Moriyama and Kawano, 2003). Similar selective pressure has been
21 suggested as a driving force for the evolution of multiple mating types in fungi without mobile
22 gametes (Hurst, 1995).

23 The evolutionary stability of two mating types can therefore be explained by the high long-term
24 fitness cost of biparental inheritance in the newly invading gamete class compared to the advantage
25 of having more compatible mating partners. On the other hand, the results show that a third mating
26 type allele invades much more readily in tandem with a new allele at the mitochondrial-inheritance
27 locus U_{m1} coding for a novel mitochondrial recognition and destruction machinery. In this case,
28 unions between all three gamete types remain uniparental throughout the invasion. Stable
29 population of three mating types at equal frequencies can therefore become established even at
30 low gamete mortality rates δ; the same remains true for subsequently invading mating-type alleles.
31 Under these assumptions the number of mating types in the population would seem to be limited
32 only by the amount of distinct molecular mechanisms controlling the transmission of organelle
33 genomes. This scenario, however, is critically dependent on the simultaneous invasion of two
34 novel mutations at linked loci, both controlling vastly complex processes of mate recognition and
35 mitochondrial destruction, and is therefore highly unlikely.

36 How does the present analysis compare to the previous work suggesting that mating types could
37 have evolved together with the uniparental inheritance of cytoplasmic genes? In the model of Hurst
38 and Hamilton (1992), gamete classes evolve to eliminate the conflict between cytoplasmic genes
39 inherited from distinct parents (Hurst, 1995). Similarly, Hutson and Law (1993) considered the

spread of "selfish" endosymbionts with fixed fitness costs. While multiple cases of selfish organelles and genetic elements are known (Schable and Wise, 1998; Taylor et al., 2002; Clark et al., 2012), it is unlikely that they occur at high enough frequencies to account for the universality of mating types with uniparental transmission of cytoplasm in eukaryotes. Selection against deleterious mitochondrial mutations, on the other hand, provides a more general explanation. While the rates of mtDNA mutation accumulation vary substantially between groups, in many cases they can exceed the evolution rates of nuclear genes by an order of magnitude, supporting the hypothesis (Lynch, 2006). Given the central role of mitochondria in eukaryotic metabolism, it is not surprising that mechanisms facilitating removal of deleterious mitochondrial mutations are selected for, e.g. germline bottlenecks or early germline sequestration in bilaterians. The evolution of two mating types with uniparental organelle transmission might therefore be a consequence of the fundamental requirement for high mitochondrial quality in complex eukaryotes.

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1   **Figure legends**

2
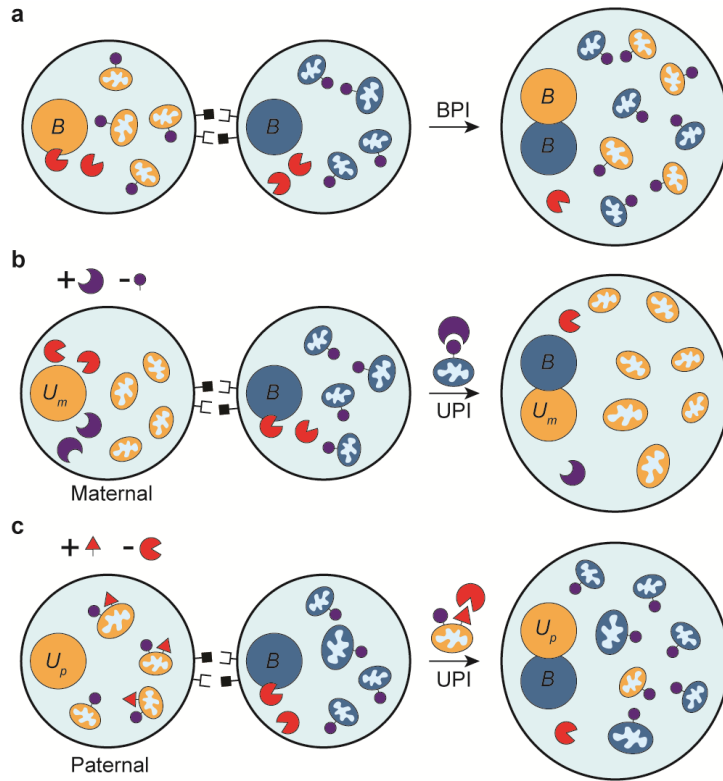
5   **Figure 1**. Two modes of uniparental inheritance differ in the way organelle inheritance is
6   controlled. In an ancestral state, mitochondrial surface proteins do not have corresponding nuclear-
7   coded molecular factors targeting the organelles for destruction (**a**). Maternal mode of UPI arises
8   with one of the gametes developing the ability to recognize the mitochondrial marker protein
9   universal to the whole population, while protecting its own organelles e.g. by removing the tag
10   (**b**). Similarly, in paternal mode of UPI, one of the gametes marks its mitochondria with a new
11   universally recognizable marker, but loses the ability to target it for destruction in its own
12   cytoplasm (**c**). Alternatively, the paternal gamete can simply remove a part of its own organelle
13   population before fertilization.
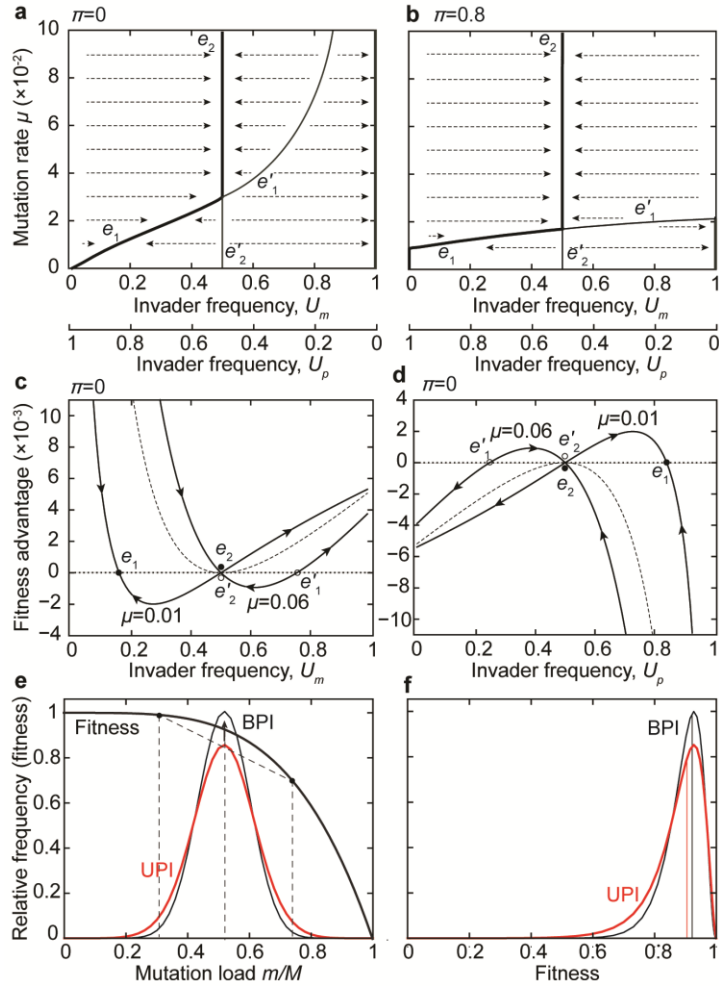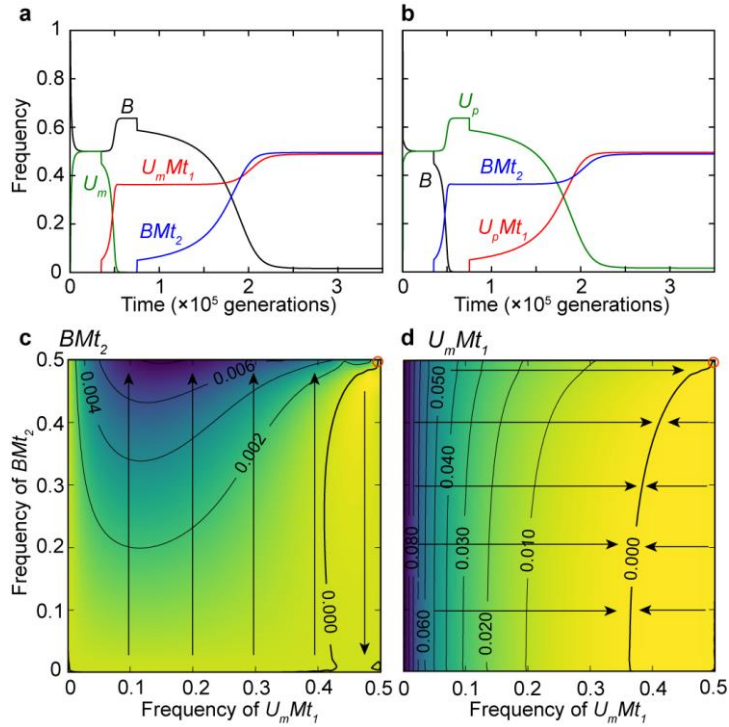
Figure 2. Invasion of the uniparental inheritance alleles in a population without mating types. Equilibria for the maternal (U_m) and paternal (U_p) UPI alleles for strict uniparental inheritance π=0 (**a**) and asymmetric inheritance with mitochondrial leakage π=0.8 (**b**). Equilibria e_1 and e_2 (thick lines) are stable, while e_1' and e_2' indicate unstable states. Fitness advantage of the UPI mutants U_m (**c**) and U_p (**d**) with π=0 depends on their frequency, explaining the stability of equilibria in (**a**). Dashed line indicates the fitness landscape at the saddle point $\mu = 0.03$, where equilibria e_1 and e_2 coincide. The strength of epistasis $\xi = 2$, $M = 50$. The asymmetric inheritance increases variance in the mutation load allowing for more efficient purifying selection and giving a long-term advantage, while mitochondrial mixing under the biparental inheritance (BPI) increases the frequency of intermediate cytoplasmic states (**e**). But with negative epistasis between mitochondrial mutations (**e**), mitochondrial mixing results in higher fitness than would be expected under linear fitness interactions (**f**), giving BPI a short-term advantage.

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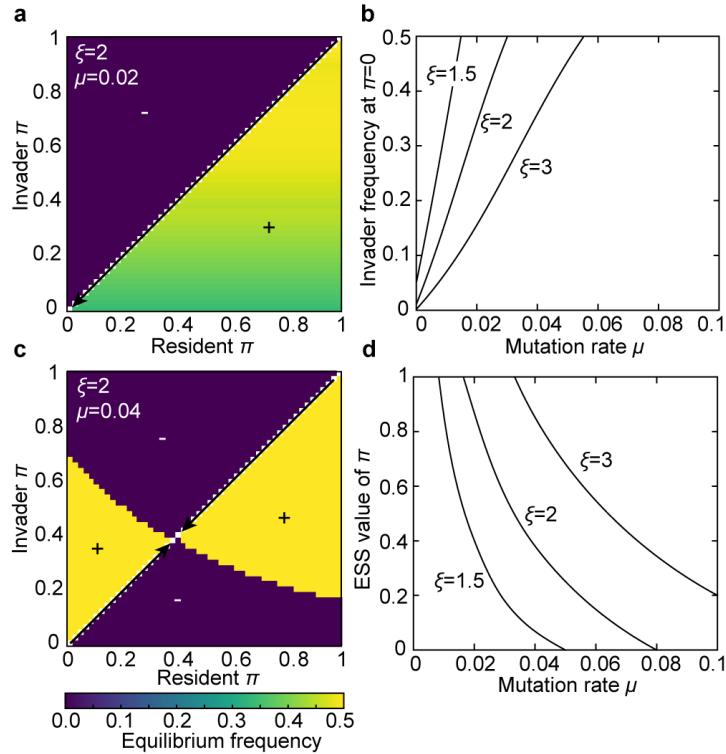
Figure 3. Evolutionary stability of asymmetric cytoplasmic inheritance in a population without mating types. Pairwise invisibility plot shows that with maternal control of cytoplasmic inheritance, the only asymmetric ESS is a strict UPI, i.e. $\pi=0$ (**a**), with the frequency of U_m at the ESS increasing with higher mutational load (**b**). In contrast, with paternal control the ESS can lie anywhere between the fully symmetric and strict uniparental inheritance (**c**), depending on mutation rates and epistatic interactions (**d**).

Figure 4. Invasion of self-incompatibility alleles Mt_1 and Mt_2 linked to the mitochondrial inheritance locus U_m/B (U_p/B). Due to the higher rate of uniparental gamete unions, U_mMt_1 (or BMt_2 assuming the paternal control) replaces the universally compatible allele U_m (B), but to a lower frequency (**a**-**b**). Similarly, the self-incompatible BMt_2 (U_pMt_1 under paternal regulation) then increases in frequency at the expense of B (U_p), as it makes the asymmetric unions more frequent. The fitness advantage of the invading BMt_2 (U_pMt_1) only increases with its frequency (**c**). The fitness advantage of U_mMt_1 (BMt_2) over B (U_p) also increases as BMt_2 (U_pMt_1) invades, as less frequent biparental gamete unions reduce the short-term fitness advantage of the universal resident B (**d**). Arrows indicate the expected direction of evolution in the population consisting of U_mMt_1, BMt_2 and B. Parameter values are $\xi = 1.5$, $\mu = 0.04$, $\pi = 0.4$, $\delta = 10^{-5}$.

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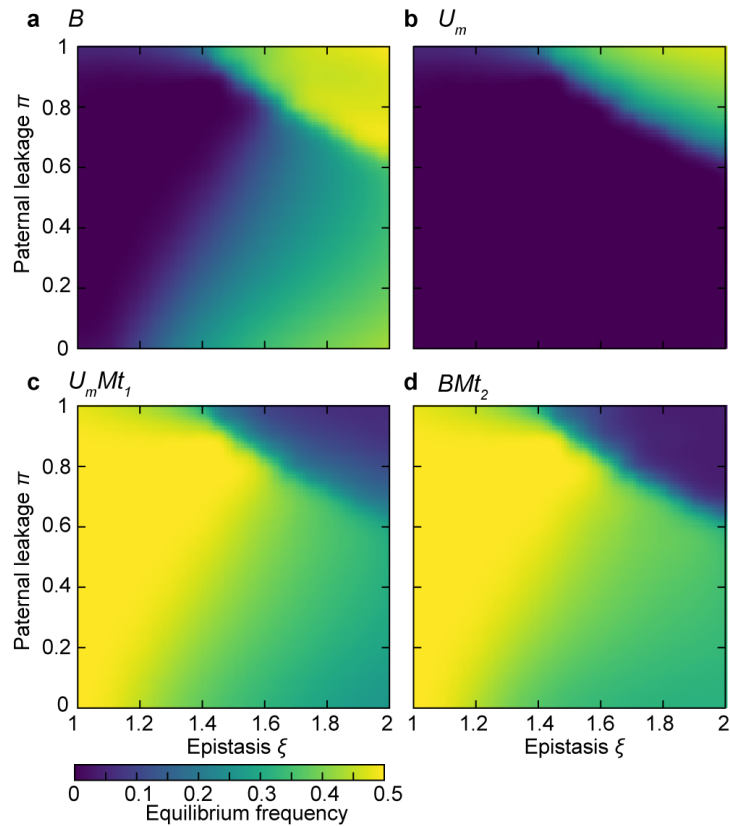
Figure 5. Equilibrium genotype frequencies for *B*, U_m, U_mMt_1 and BMt_2 (U_p, *B*, BMt_2 and U_pMt_1 under paternal inheritance regulation) after the invasion of both mating-type alleles linked to the mitochondrial inheritance loci, as a function of paternal leakage π and the strength of epistasis between mitochondrial mutations ξ. Self-incompatible mating types reach fixation under weak epistatic interactions, where the long-term fitness advantage of UPI is maximized. With stronger epistasis, the fixation of mating types is facilitated by the intermediate paternal leakage, implying the importance of paternal control of mitochondrial inheritance. Under strong negative epistasis and significant paternal leakage, mating types do not replace the universally compatible gametes due to weak long-term variance effects. Gamete death rate is set to $\delta = 0.001$, the mitochondrial mutation rate is $\mu = 0.03$.
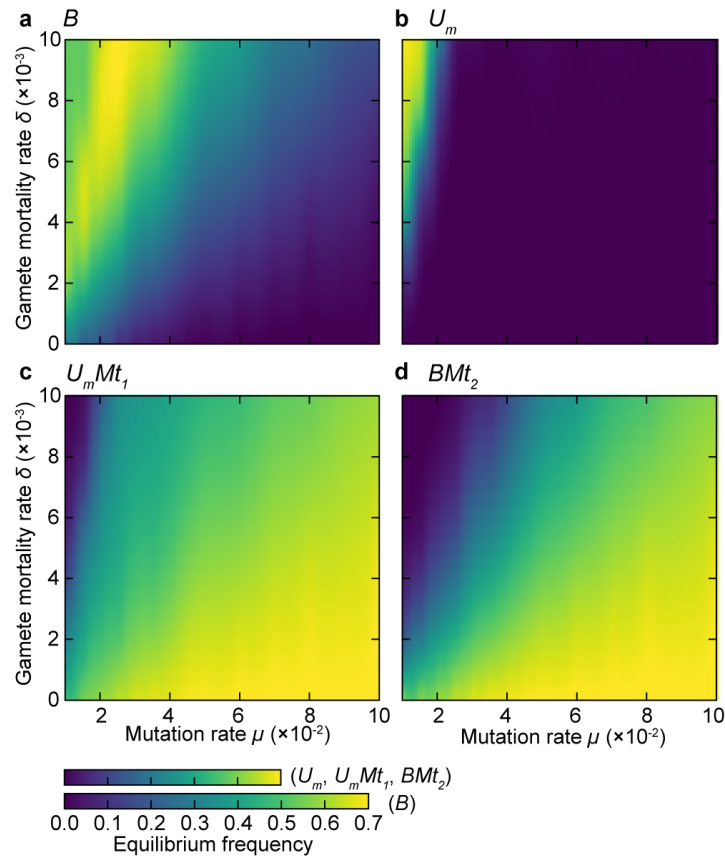
Figure 6. Equilibrium frequencies of genotypes B, U_m, U_mMt_1 and BMt_2 (U_p, B, BMt_2 and U_pMt_1 under paternal inheritance regulation) after the invasion of both mating-type alleles linked to the mitochondrial inheritance loci, as a function of gamete mortality rate δ and mitochondrial mutation rate μ. Self-incompatible gamete classes replace universal gamete types under high mutation rates and low gamete mortality rates. The strength of epistasis is $\xi = 1.4$, the rate of paternal leakage of mitochondria is set to $\pi = 0.2$.