

EVOLUTION OF HIERARCHICAL CYTOPLASMIC INHERITANCE IN THE PLASMODIAL SLIME MOLD *PHYSARUM POLYCEPHALUM*

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Abstract.—A striking linear dominance relationship for uniparental mitochondrial transmission is known between many mating types of plasmodial slime mold *Physarum polycephalum*. We herein examine how such hierarchical cytoplasmic inheritance evolves in isogamous organisms with many self-incompatible mating types. We assume that a nuclear locus determines the mating type of gametes and that another nuclear locus controls the digestion of mitochondria DNAs (mtDNAs) of the recipient gamete after fusion. We then examine the coupled genetic dynamics for the evolution of self-incompatible mating types and biased mitochondrial transmission between them. In *Physarum*, a multiallelic nuclear locus *matA* controls both the mating type of the gametes and the selective elimination of the mtDNA in the zygotes. We theoretically examine two potential mechanisms that might be responsible for the preferential digestion of mitochondria in the zygote. In the first model, the preferential digestion of mitochondria is assumed to be the outcome of differential expression levels of a suppressor gene carried by each gamete (suppression-power model). In the second model (site-specific nuclease model), the digestion of mtDNAs is assumed to be due to their cleavage by a site-specific nuclease that cuts the mtDNA at unmethylated recognition sites. Also assumed is that the mtDNAs are methylated at the same recognition site prior to the fusion, thereby being protected against the nuclease of the same gamete, and that the suppressor alleles convey information for the recognition sequences of nuclease and methylase. In both models, we found that a linear dominance hierarchy evolves as a consequence of the buildup of a strong linkage disequilibrium between the mating-type locus and the suppressor locus, though it fails to evolve if the recombination rate between the two loci is larger than a threshold. This threshold recombination rate depends on the number of mating types and the degree of fitness reduction in the heteroplasmic zygotes. If the recombination rate is above the threshold, suppressor alleles are equally distributed in each mating type at evolutionary equilibrium. Based on the theoretical results of the site-specific nuclease model, we propose that a nested subsequence structure in the recognition sequence should underlie the linear dominance hierarchy of mitochondrial transmission.

Key words.—Cytoplasmic inheritance, heteroplasmy, linear dominance hierarchy, *Physarum polycephalum*, preferential digestion of mitochondria, self-incompatible mating type.

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Uniparental transmission of organelles is a ubiquitous phenomenon in eukaryotes. The transmission of organelle genes from one parent to the offspring can be blocked at any stage of sexual reproduction (Birky 1995, 2001): organelle DNA of the recipient sex can be degraded during gametogenesis, it can be shed from gametes before fertilization or may fail to enter the egg during fertilization, and it can be selectively degraded in the zygotes after fertilization. In the mammals, the sperm mitochondria are ubiquitinated during epididymal passage. This ubiquitination causes elimination after the sperm mitochondria encounter the egg's cytoplasmic destruction machinery (Sutovsky et al. 1999, 2001, 2002). In green alga *Chlamydomonas reinhardtii*, the uniparental transmission of chloroplast DNA (cpDNA) is ascribed to the selective digestion of unmethylated recipient cpDNA. The fact that there are several different factors that may prevent organelles from being biparentally transmitted would suggest that uniparental organelle transmission is a stable evolutionary outcome that has been attained and maintained by natural selection. In this paper we focus on what kind of relationship is built up between the self-incompatible mating type and cytoplasmic inheritance.

Although anisogamy is thought to play a critical role in uniparental organelle inheritance, such inheritance is by no means rare in isogamous species. For example, the cpDNA is transmitted only from one mating type of the isogamous green algae *Chlamydomonas reinhardtii*. The molecular mechanism of this uniparental inheritance has been recently clar-

ified (Burton et al. 1979; Royer and Sager 1979; Sano et al. 1981; Nishimura et al. 1999; Umen and Goodenough 2001; Nishiyama et al. 2002). In the isogamous alga *Chlamydomonas* with two mating-types, mt^+ and mt^- , the cpDNA is transmitted only from mt^+ alga. When two gametes of opposite mating types fuse, a signal is sent to activate a mt^+ nuclear gene. The synthesized protein then activates a nuclease, which preferentially digests the cpDNA from the mt^- gamete. Nuclear genes *Mta1* and *Ezy2* participate in the protection and destruction of cpDNA, respectively (Patrick et al. 2002). Experimental studies have shown that the mt^+ cpDNA is methylated before the gametic fusion and this protects against the nuclease. Discouraging evidence against the methylation hypothesis is that uniparental mitochondria transmission was found not to be blocked by the treatment of the zygote with the methylation inhibitor 5-aza-2'-deoxycytidine (Nishimura et al. 1999). As Nishimura et al. (1999) pointed out, however, methylation may occur prior to zygote formation and evidence supporting the methylation hypothesis has accumulated since then (Umen and Goodenough 2001; Nishiyama et al. 2002). The most striking example of uniparental organelle transmission in isogamous species can be found in myxameba of the plasmodial slime mold, which is known to have many mating types. For any pair of compatible mating types, mitochondria are inherited from only one parent. A striking fact in the uniparental transmission of *Physarum* is that all gametes can act as either donors or recipients of mitochondria—it completely depends on the

mating type of the mate. When the unidirectional mitochondrial inheritance rules are tabulated, it is found that they constitute a linear dominance hierarchy in *Physarum* mating types.

The mechanisms for uniparental organelle transmission have been studied so far in systems of only two mating types. The situation becomes significantly more complex if there are more than two mating types (as in *Physarum*), and no theoretical work has been done for such a system. The aim of this paper is, therefore, to examine what is responsible for the evolution and the maintenance of this striking linear hierarchy system in the organelle donor-recipient relationship between *Physarum* mating types.

There are two phases in the life cycle of plasmodial slime mold *Physarum polycephalum*, the haplophase myxamoeba and the diplophase plasmodium (see Kawano et al. 1995). The spores hatch to release myxamoeba isogametes. Individual isogametes of different mating types then pair to form a diploid zygote. The zygote develops into a giant syncytium called the plasmodium and then into lobed sporangia. The life cycle is completed by the meiosis within spores.

In this paper, we focus on an interesting phenomenon found in *P. polycephalum*: a hierarchical rank order relationship between mating types of myxamoeba isogametes in their directional mitochondrial inheritance. The myxamoeba isogametes have many self-incompatible mating types, and any pair of different mating types can fuse and form a zygote. Just after the fusion of two isogametes, the zygote contains mitochondria from both gametes. However, mitochondria from one parental gamete are finally eliminated from the zygote. This uniparental cytoplasmic inheritance is found in any pair of mating types in *P. polycephalum*, and all the mating types can be linearly ordered according to the direction of cytoplasmic inheritance.

The mating types of *Physarum* gametes (myxamoeba) are determined by three nuclear loci, *matA*, *matB*, and *matC* (Youngman et al. 1979; Kawano et al. 1987b). There are at least 15 alleles at each *matA* and *matB* locus, and three alleles at the *matC* locus. Thus, there are at least 675 self-incompatible mating-types in *P. polycephalum*. Along with mating-type determination, the *matA* locus has another function: the control of directional mitochondrial inheritance (Kawano et al. 1987a). According to extensive crossing tests of myxamoeba, Kawano and his colleagues found the following ordering in the direction of mitochondria transmission between *matA* genotypes of gametes: *matA7* > *matA2* > *matA11* > *matA12* > *matA1/matA15* > *matA6*, where the relative order of *matA1* and *matA15* remains undetermined, as they have not been tested against each other (Kawano and Kuroiwa 1989; Kawano et al. 1995). Though at least 15 alleles are known at the *matA* locus, only six mating types have been tested for their directions in mitochondria inheritance. When two gametes with *matA7* and *matA2* fuse, for example, the donor of mtDNA is the *matA7* gamete. The *matA2* gamete contributes no mtDNA in this mating pair. However, the same mating-type *matA2* becomes the mtDNA donor in the mating with *matA11*.

Though the molecular mechanism of mitochondrial DNA (mtDNA) degradation in zygotes to have such mating type genes is not yet known, mitochondria of the recipient mating

type are selectively degraded in the *Physarum* zygote (Meland et al. 1991) as in the uniparental inheritance of the *Chlamydomonas* chloroplast. We assume in our model that there is a nuclear locus that is responsible for the selective elimination of the mtDNA of the recipient gamete. We call this the "suppressor" locus, as it suppresses the mitochondrial transmission from the other gamete. In our model, the suppressor locus may or may not be genetically linked to the mating-type locus, though in *Physarum* these two loci are completely linked (i.e., the suppression of mitochondria transmission of the other gamete is controlled by the same locus, *matA*, that determines the mating-type of the gamete). We examine how the recombination rate between loci affects the evolutionary outcome.

We consider two possible mechanisms by which the degradation of mtDNAs of recipient gamete is achieved. In both models, we examine how the linear dominance hierarchy of uniparental mitochondria transmission evolves in an isogamous organism with many mating types, where the mating type of an isogamete is determined by a multiallelic nuclear locus (the mating type locus), and the digestion of mitochondria of the recipient is controlled by another multiallelic nuclear locus (the suppressor locus). We focus on how the number of mating types, the degree of fitness reduction in a heteroplasmic zygote, and the linkage between the mating type and the suppressor loci influence the evolutionary outcome.

SUPPRESSION-POWER MODEL

To study the evolution of hierarchical cytoplasmic inheritance, we here consider the population of an isogamous organism and assume that the mating-type of gametes and the digestion of cytoplasm of the recipient gamete after fusion are under the control of two nuclear loci. We assume that there are n alleles, M_1, M_2, \dots, M_n , in the mating-type locus M , and that two gametes can mate if they carry different mating type alleles M_i and M_j with $i \neq j$. We also assume that there are n alleles, S_1, S_2, \dots, S_n , in the suppressor locus S that differ in their gene expression levels, these levels being arranged in the descending order of their gene expression levels so that the S_1 allele has the greatest expression level and S_n has the least. If fused gametes have different suppressor alleles S_i and S_j with $i < j$, we assume that the mtDNAs from the gamete carrying the S_j suppressor allele with a lower expression level are digested in the zygote, ensuring the uniparental transmission of mtDNA from the S_i -carrying gamete. If two gametes carry the identical suppressor allele ($i = j$), then the mitochondrial inheritance will be biparental, that is, the zygote is heteroplasmic. We assume that the heteroplasmic zygote suffers fitness reduction by the amount α , which we call the "cost of heteroplasmy," and we call the phenomena "heteroplasmic depression." Though often assumed in theoretical papers (e.g., Cosmides and Tooby 1980; Hurst and Hamilton 1992), there has been no quantitative measurement of the cost of heteroplasmy. We therefore varied the degree α of the heteroplasmic depression (the cost of heteroplasmy) from a very weak value ($\alpha \approx 0$) to a very large one ($\alpha = 0.5$, semilethal). By contrast, there is clear evidence for the overdominance in the *matA* (mating-

type) locus. The *matA* homozygotes remain ameoboid and hence fail to differentiate further (Kawano et al. 1995).

We denote the frequency of gamete genotype $M_i S_k$ by p_{ik} ($i, k = 1, 2, \dots, n$). By definition, $\sum_{i=1}^n \sum_{k=1}^n p_{ik} = 1$. It is convenient to define the frequency x_i of the mating-type allele as M_i : $x_i = \sum_{k=1}^n p_{ik}$, and the frequency y_k of the suppressor allele as S_k : $y_k = \sum_{i=1}^n p_{ik}$. The change in the gamete frequencies in one generation is calculated by dividing the life cycle of the organism into four steps. (1) gamete fusion: two isogametes are chosen randomly from the gamete pool. If a randomly chosen pair of gametes carries different mating-type alleles, they fuse to form a zygote; if they carry the same mating-type allele, both of them are discarded from the population. (2) Viability selection: zygotes are subject to viability selection due to the conflict between biparentally transmitted cytoplasm. If the zygote formed in the first step has the identical suppressor allele, the zygote survivorship is reduced by the amount of the α because of heteroplasmic depression. (3) Recombination: two nuclear loci M and S may undergo recombination during meiosis. (4) Gametogenesis: the gametes in the next generation are then produced by gametogenesis. Combining the frequency changes during these four steps, we can derive recursion equations for the change in the frequency p_{ik} of gamete genotype $M_i S_k$ in one generation (see Appendix 1):

$$p'_{ik} = (W_{ik} p_{ik} - r D_{ik}) / \bar{W}. \tag{1a}$$

where

$$W_{ik} = 1 - x_i - \alpha(y_k - p_{ik}) \tag{1b}$$

is the fitness of gamete genotype $M_i S_k$, and

$$\begin{aligned} \bar{W} &= \sum_{i=1}^n \sum_{k=1}^n W_{ik} p_{ik} \\ &= 1 - \sum_{i=1}^n x_i^2 - \alpha \sum_{k=1}^n y_k^2 + \alpha \sum_{i=1}^n \sum_{k=1}^n p_{ik}^2 \end{aligned} \tag{1c}$$

is the population mean fitness, r is the recombination rate between the mating-type locus and the suppressor locus, and $D_{ik} = p_{ik} - x_i y_k$ is the linkage disequilibrium. The fitness form (1b) has a clear biological meaning. The fitness W_{ik} of gamete genotype $M_i S_k$ decreases with the increased frequency x_i of the same mating type M_i in the population, because the chance is greater for a randomly chosen gamete to have the same mating-type allele (the rare sex advantage in finding suitable mates; Wright 1939; Iwasa and Sasaki 1987). Similarly, the greater the frequency y_k of the suppressor allele S_k , the greater the mean fitness reduction due to heteroplasmy by biparental mitochondrial transmission, because the gamete more often mates with one carrying the same suppressor allele. As the gametes of the same mating type never fuse, the fitness reduction by heteroplasmic depression is proportional not to y_k but to $\sum_{j \neq i} p_{jk} = y_k - p_{ik}$, the frequency of allele S_k in gametes having different mating-type alleles.

There are three classes of equilibria of the dynamics (1a-c). The first class of equilibria is the isoplethy equilibrium in which the frequencies are the same for all genotypes:

$$\hat{p}_{ik} = 1/n^2. \tag{2}$$

The second and third classes of equilibria are stable and

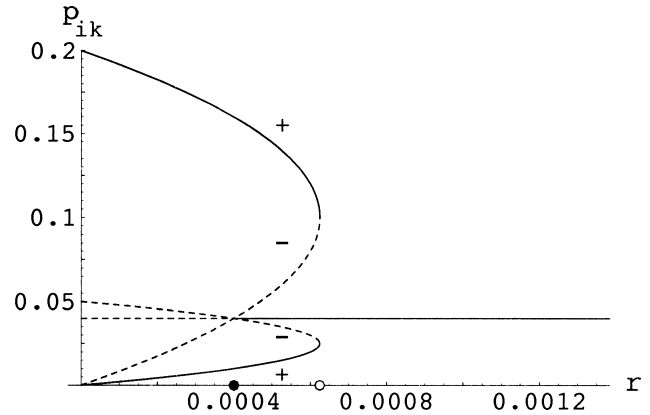


FIG. 1. Bifurcation diagram of model (1). The stable (solid) and unstable (dashed) branches of equilibria are plotted against the recombination rate r between the mating-type locus and the suppressor locus. There are two threshold recombination rates. If r is smaller than $r_c^{(1)} = \alpha/n^2$ (filled circle), the population converges to one of the equilibria in which each suppressor allele is exclusively localized in a different mating type (p_{ik} converges to the upper + branch if the suppressor S_k is to be localized in the mating type M_i and otherwise converges to the lower + branch). If r is larger than $r_c^{(2)} = \alpha/4(n - 1)$ (open circle), the population converges to the isoplethy equilibrium (the line parallel to r axis). If r is in between two thresholds, the population converges to either the isoplethy equilibrium or one of the asymmetric equilibria, depending on the initial condition. Parameters: $n = 5, \alpha = 0.01$.

unstable asymmetrical equilibria with strong linkage disequilibrium between mating-type locus and suppressor locus, by which the suppressor alleles are mutually exclusively localized in different mating types. There are $n!$ pairs of stable and unstable asymmetric equilibria, this multiplicity being due to permutation with regard to the mapping between a mating type and the dominant suppressor allele in each mating type. More specifically, a pair of stable (+) and unstable (-) asymmetrical equilibria in which the suppressor allele S_{k_i} is predominant in the mating type M_i ($i = 1, 2, \dots, n$) are given by

$$p_{ik_i}^{*(\pm)} = \frac{1}{2n} \left[1 \pm \sqrt{1 - \frac{4(n-1)r}{\alpha}} \right], \tag{3a}$$

$(i = 1, 2, \dots, n) \text{ and}$

$$p_{ik}^{*(\pm)} = \frac{1}{2n(n-1)} \left[1 \mp \sqrt{1 - \frac{4(n-1)r}{\alpha}} \right], \tag{3b}$$

$(i, k = 1, 2, \dots, n; k \neq k_i)$

where (k_1, k_2, \dots, k_n) is a permutation of $(1, 2, \dots, n)$. See Appendix 2 for the derivation of (3a, b).

According to the local stability analysis of the isoplethy equilibrium (2) in Appendix 3, we establish the following results (Fig. 1). First, the isoplethy equilibrium is locally stable if the recombination rate r between the mating type and the suppressor locus is larger than the threshold:

$$r_c^{(1)} = \alpha/n^2. \tag{4}$$

Second, when the isoplethy equilibrium loses local stability for a recombination rate smaller than $r_c^{(1)}$, an initial small

deviation from the equilibrium leads to the growth of linkage disequilibrium D_{ik} values between loci, indicating that nonrandom association between a mating-type allele and a suppressor allele has been established in the population. Indeed, the population converges to an asymmetrical equilibrium (3a, b) with a strong linkage disequilibrium, by which each one-to-one nonrandom association between a mating-type M_i and a suppressor allele S_{k_i} is realized. Finally, asymmetric equilibria are locally stable if r is smaller than the second threshold:

$$r_c^{(2)} = \alpha/4(n - 1). \quad (5)$$

The second threshold is derived from the condition in which the stable and unstable branch for the equilibrium frequency of dominant suppressor coincide with each other ($p_{ik_i}^{*(+)} = p_{ik_i}^{*(-)}$). If r is in between $r_c^{(1)}$ and $r_c^{(2)}$, the population converges to either the isoplethy equilibrium or one of the asymmetric equilibria, depending on the initial condition. $r_c^{(1)}$ and $r_c^{(2)}$ coincide if $n = 2$ (two mating types), but $r_c^{(1)} < r_c^{(2)}$ for $n \geq 3$, and hence a region of bistability exists if there are more than two mating types.

These results illustrate how a linear dominance hierarchy of uniparental cytoplasmic transmission is established and maintained in isogamous organisms with many self-incompatible mating types. If the linkage between the mating-type locus and the suppressor locus is sufficiently tight, $r < r_c^{(1)} = \alpha/n^2$, small deviation from the uniform distribution of suppressors over mating types grows in the population and eventually a strong linkage disequilibrium between mating-type locus and suppressor locus is built up. Some mating types are then tightly associated with strong suppressors, others with weak suppressors, and still others with intermediate suppressors, making the first the organelle donors, the second recipients, and the last either donors or recipients depending on the mating type they mate. Once the linear hierarchy is established, the condition for its maintenance becomes less stringent: $r < r_c^{(2)} = \alpha/4(n - 1)$.

To conclude, the dominance hierarchy can become established by the buildup of nonrandom association between mating-type alleles and suppressor alleles. However, the linkage between the mating-type locus and the suppressor locus must be very strong for the establishment of the linear hierarchy when the number n of mating types is large (e.g., with the assumption $\alpha = 0.01$, r must be smaller than 0.0044 for its new establishment and must be smaller than 0.018 for its maintenance, if the number n of mating-type alleles is as large as 15, as in *Physarum matA* locus).

These results are confirmed by the numerical simulations of (1). When two loci are completely linked ($r = 0$), each mating type will be entirely occupied by a different suppressor allele (Fig. 2a). The same, but less extreme, result follows when the recombination rate is positive but smaller than the threshold—a strong, nonrandom association will be established in the equilibrium population, but rare suppressor alleles are maintained by recombination at low frequencies in each mating type. If, however, the linkage is too loose, the population converges to the isoplethy equilibrium. Figure 2b illustrates the trajectory in which the population converges to the isoplethy equilibrium. The results remain qualitatively the same when we change the degree α of heteroplasmic

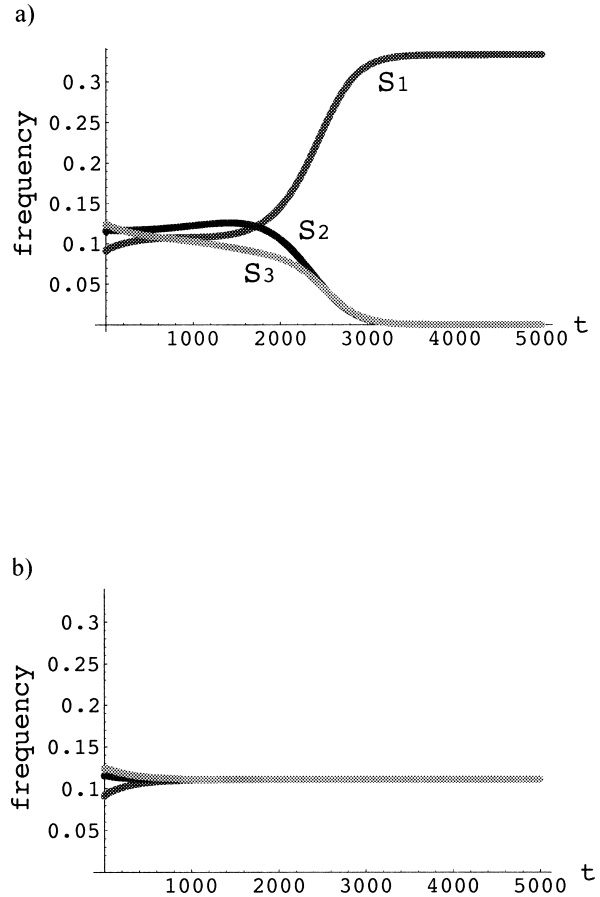


FIG. 2. Change of allele frequencies of the suppressor alleles S_1 , S_2 , and S_3 in the mating type M_1 in model (1) starting with random initial frequencies. There are three (n) mating-type alleles suppressor alleles, and the cost of heteroplasmy α is 0.01. The suppressor frequencies in the other mating-types (M_2 and M_3) change similarly, though the suppressor allele that will be fixed (a) or will dominate (b) is different. (a) The mating-type locus and the suppressor locus are completely linked ($r = 0$). (b) The recombination rate is 0.01, which is greater than either of the two thresholds ($r_c^{(1)} \approx 0.00111$ and $r_c^{(2)} = 0.00125$).

depression as long as it is positive, though the threshold recombination rates (4) and (5) change with changing α , and it takes longer for the convergence to an equilibrium when α is small.

SITE-SPECIFIC NUCLEASE MODEL

Now we proceed to a biologically more concrete model for the evolution of linear dominance hierarchy for uniparental mitochondria transmissions in isogamous organisms with many mating types. As mentioned before, we here assume that the postmating digestion of mtDNA is performed by the site-specific nuclease carried by each gamete, which cut the DNA at its unmethylated recognition sites. The mtDNAs in each gamete are methylated at the same recognition sites before fusion and are thereby protected against the nuclease of the same gamete. The suppressor alleles convey information on the recognition sequence of nuclease and methylase.

As before, the mating type of gamete is determined by a nuclear locus with n alleles (M_1, \dots, M_n). Alleles at another nuclear locus (the suppressor locus) convey the information for the recognition sequences of the nuclease and methylase. For simplicity, we assume that binary sequences of up to a certain prefixed maximum length L constitute the recognition sequences σ_j values encoded by different suppressor alleles. For example, if $L = 5$, $\sigma_0 = 0$, $\sigma_1 = 1$, $\sigma_2 = 00$, \dots , $\sigma_{m-1} = 11111$, where $m = 2 + 2^2 + \dots + 2^5$ is the total number of suppressor alleles. The zygotic fitness w_{kl} is determined by two suppressor alleles, S_k and S_l , from each gamete as:

$$w_{kl} = \begin{cases} 1 & \text{if } \sigma_k \subset \sigma_l \text{ or } \sigma_l \subset \sigma_k \\ 1 - \alpha & \text{if } \sigma_k = \sigma_l \\ 0 & \text{if otherwise,} \end{cases} \quad (6)$$

where $\sigma \subset \tau$ indicates that σ and τ are in a nested relationship, that is, the sequence σ is a subsequence of τ . If one of the recognition sequences of suppressors is a subsequence of the other, the mtDNAs of one parent are protected against both nucleases carried by gametes, but that from the other parent are not, leading to uniparental mitochondria transmission. In such case, there is no fitness reduction in the zygote (the first case in eq. 6). If two suppressors are identical, all mitochondria are protected against either of the nucleases, leading to biparental transmission of mitochondria, thereby causing the fitness reduction of the zygote by α due to heteroplasmic depression (the second case). Finally, if the recognition sequences are not nested or identical, nucleases from one parent digest the mtDNAs from the other parent. If this happens, all mtDNAs are eliminated from the zygote, leading to lethality of the zygote (the last case).

The recursion for the change in the frequency p_{ik} of gamete genotype $M_i S_k$ is then

$$p'_{ik} = \frac{(1-r) \sum_{j \neq i} \sum_l w_{kl} p_{ik} p_{jl} + r \sum_{j \neq i} \sum_l w_{kl} p_{il} p_{jk}}{\bar{w}}, \quad (7)$$

where $\bar{w} = \sum_i \sum_{j \neq i} \sum_k \sum_l w_{kl} p_{ik} p_{jl}$ is the mean fitness of the population, and r is the recombination rate between the mating type and the suppressor loci. The recursion (7) can be rewritten in the same form as (1a) by replacing W_{ik} defined in (1b) by

$$\tilde{W}_{ik} = W_{ik} - \sum_{j(\neq i)} \sum_{l(-k)} p_{jl}, \quad (8a)$$

D_{ik} being defined in (1c) by

$$\tilde{D}_{ik} = D_{ik} + \left[p_{ik} \sum_{l(-k)} y_l - y_k \sum_{l(-k)} p_{il} \right], \quad (8b)$$

and \bar{W} being defined in (1c) by

$$\bar{\tilde{W}} = \bar{W} - \sum_i \sum_{j(\neq i)} \sum_k \sum_{l(-k)} p_{ik} p_{jl}, \quad (8c)$$

where the relationship $k \sim l$ indicates that two gametes carrying the suppressor alleles S_k and S_l are mutually destructive (the last case in eq. 6, σ_k and σ_l are neither identical nor nested). From this rewritten form, it is clear that if no pair of suppressor alleles segregating in the population show such

a mutually destructive relationship (i.e., $\forall k, l; k \not\sim l$), then the model (7) is reduced to the previous model (1).

Strong selection against any pair of incompatible (mutually destructive) suppressor alleles in the model (7) indeed quickly purges out the incompatible pairs from the population, leaving only suppressor alleles such that their recognition sequences constitute a nested recognition subsequence structure (e.g., $1 \subset 10 \subset 010 \subset 0100 \subset 10100$). Figures 3 and 4 show the result of numerical simulations with $L = 5$ and illustrate how this purging of mutually destructive pairs and the buildup of linkage disequilibrium proceed over time. In both examples assuming five mating types, the initial population harbors 62 ($= 2 + 2^2 + \dots + 2^5$) suppressor alleles differing in their recognition sequences (binary sequences of up to length 5). There are many mutually destructive pairs of gametes in the initial population, but the population evolves so as to allow only uniparental (one-way destruction) or biparental (no destruction) mitochondrial transmissions between randomly chosen gametes. That is, any suppressor allele that deviates from a nested subsequence rule (which itself is selected randomly of 32 possible combinations of alleles that yield such relationship) finally goes extinct. On this condition, the model (7) becomes equivalent to the previous model (1). The next step is the buildup of linkage disequilibrium between such a combination of suppressor alleles with nested recombination sequences and mating types that realizes, as before, the linear dominance hierarchy in the mitochondrial transmission (Fig. 5).

If the recombination rate between the mating-type locus and the suppressor locus is smaller than the threshold ($r < r_c^{(1)} = \alpha/n^2$), different suppressor alleles are localized in different mating types at equilibrium, ensuring the linear dominance hierarchy between mating types as to the direction of mitochondrial transmissions (Figs. 3, 5, 6). By contrast, if the recombination rate is above the second threshold ($r > r_c^{(2)} = \alpha/4[n-1]$), the population converges to the isoplethy equilibrium where n suppressor alleles are equally abundant in each mating type (Fig. 4).

To quantitatively study the evolution of linear dominance hierarchy, we here define an index for the degree of the linear dominance hierarchy as follows. At any given time, the mating types are ordered according to the probability that they will become mitochondria donors under random mating. Next, the following dominance index, assigned to each pair of suppressors,

$$d_{kl} = \begin{cases} 1 & \text{if } \sigma_k \subset \sigma_l \\ & (\sigma_k \text{ is a subsequence of } \sigma_l, \text{ i.e., } \sigma_k \text{ is a donor}) \\ -1 & \text{if } \sigma_l \subset \sigma_k \\ & (\sigma_l \text{ is a subsequence of } \sigma_k, \text{ i.e., } \sigma_l \text{ is a donor}) \\ 0 & \text{if otherwise} \\ & (\text{either biparental or mutually destructive}) \end{cases} \quad (9)$$

is weighted by their frequencies found in the randomly formed pair between the mating type M_i and the mating type M_j , giving the observed mean dominance index $\tilde{\beta}_{ij}^{(o)}$ of the mating type M_i to the mating type M_j :

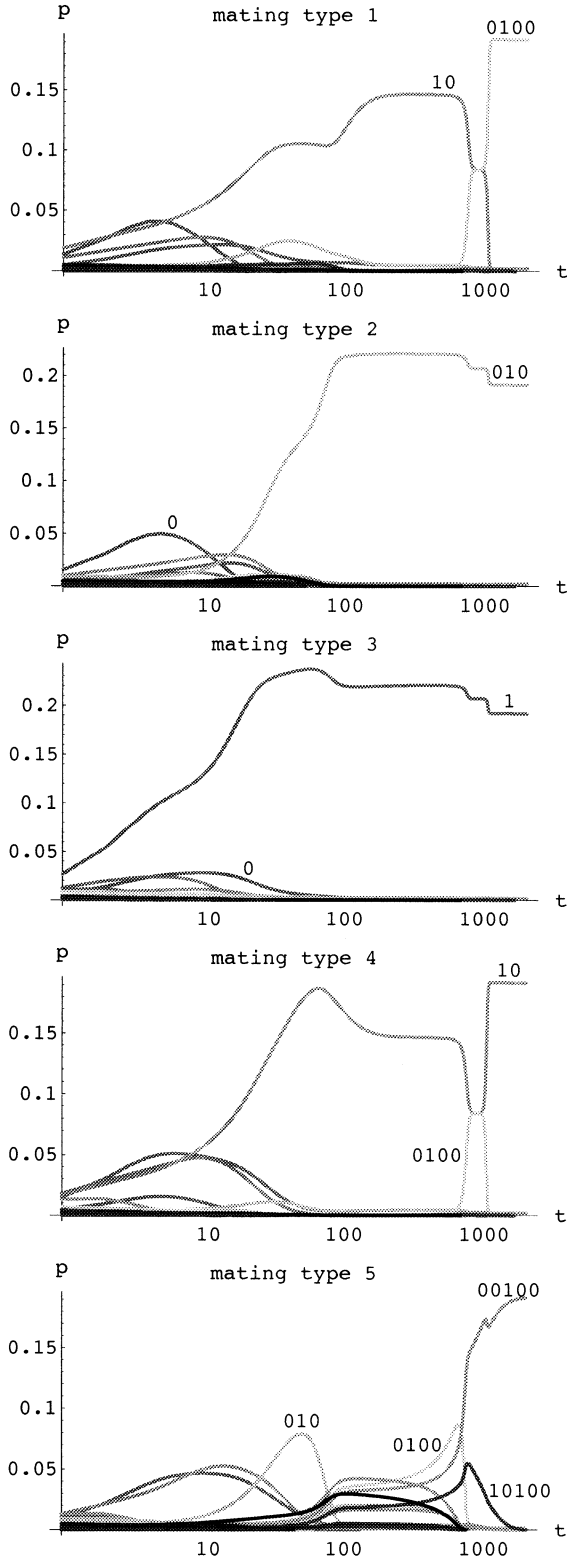


FIG. 3. Change of genotypic frequencies over time in the site-specific nuclease model with five self-incompatible mating types. The panels show the frequencies of genotypes $M_i S_j$ as a function of time in different colors for different suppressor alleles S_j , where time is logarithmically scaled. The initial population consists of 5 (mating types) \times 62 (suppressor alleles) genotypes with randomly assigned frequencies, where the suppressor alleles differ in their recognition sequence of nuclease-methylase (any binary sequence

$$\bar{\beta}_{ij}^{(o)} = \frac{\sum_k \sum_l d_{kl} P_{ik} P_{jl}}{\sum_k \sum_l P_{ik} P_{jl}}. \quad (10)$$

This is then compared with the dominance index under the perfect linear dominance relationship ($\beta_{ij}^{(e)} = 1$ if $i > j$; $= -1$ if $i < j$; note that mating types are first sorted out in an ascending order as to their donorship). The absolute difference between two transmission indices, $|\bar{\beta}_{ij}^{(o)} - \beta_{ij}^{(e)}|$, is then averaged over all pairs of mating types, and then subtracted from one, giving the index *LDI*:

$$LDI = 1 - \sum_i \sum_j |\bar{\beta}_{ij}^{(o)} - \beta_{ij}^{(e)}| x_i x_j, \quad (11)$$

where x_i is the frequency of the mating type M_i . *LDI* = 1 under a perfect linear dominance for mitochondrial transmission, and *LDI* = 0 under random relationship.

Figure 7 shows how the evolved linear dominance index *LDI* depends on the degree of heteroplasmic depression α , the recombination rate r , and the number of mating types n . The linear dominance hierarchy evolves for sufficiently large values of heteroplasmic depression (Fig. 7a, c, e), for sufficiently tight linkage between the mating type and the suppressor loci (Fig. 7b, d, f), and in either a population with three mating types ($n = 3$; Fig. 7a, b), or $n = 5$ (Fig. 7c, d), or $n = 7$ (Fig. 7e, f). Evolutionary bistability occurs in the range of recombination rate $r_c^{(1)} < r < r_c^{(2)}$, and in the corresponding range of heteroplasmic depression $\alpha_c^{(1)} < \alpha < \alpha_c^{(2)}$, where $\alpha_c^{(1)} = m^2$ and $\alpha_c^{(2)} = 4r(n - 1)$. The numerical simulation results (dots in Fig. 7) perfectly agree with the analytically derived *LDI* (curves).

We also conducted a numerical study for the basin of attraction of each locally stable equilibrium in the case of bistability. For 200 independent runs with randomly assigned initial gamete frequencies, 182 runs converged to a stable branch of asymmetrical equilibrium (3) that corresponds to a high *LDI*, 14 runs converged to the isoplethy equilibrium (2) that corresponds to *LDI* = 0, and the remaining four runs did not yet converge to any equilibrium (the parameters are $n = L = 5$, $\alpha = 0.19$, $r = 0.01$).

COMPARISON WITH THE UNCONSTRAINED SUPPRESSION NETWORK MODEL

As shown above, the linear dominance hierarchy in uniparental cytoplasmic inheritance is a robust evolutionary outcome for the relationship between self-incompatible mating

←
of length from 1 to 5 is allowed, hence giving the total number: 2 + 4 + 8 + 16 + 32 = 62). The zygote formed by random mating of different mating types is lethal if they carry mutually incompatible suppressor alleles (i.e., neither of their recognition sequences is a subsequence of the other), as the nucleases conveyed by each gamete can digest the mitochondria DNAs of the donor gamete. The linear dominance hierarchy as to the mitochondrial transmission evolves through the quasi-fixation of five suppressor alleles (labeled by their recognition sequences): 1 in mating-type 3, 10 in mating-type 4, 010 in mating-type 2, 0100 in mating-type 1, and 00100 in mating-type 5 (see also Fig. 5). The heteroplasmic depression is $\alpha = 0.5$, and the recombination rate between the mating type and the suppressor locus is $r = 0.005$.

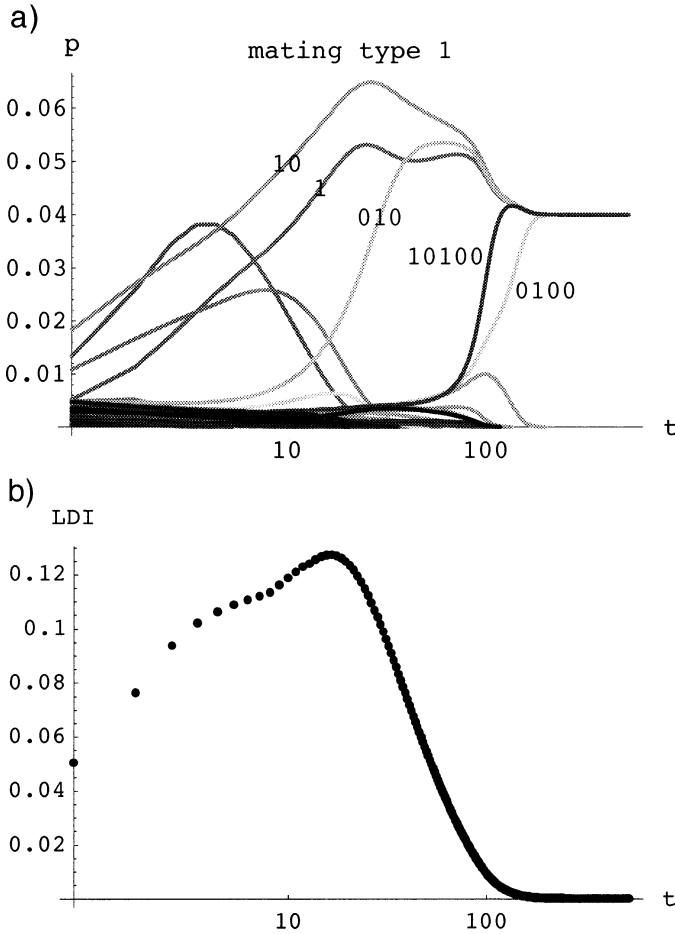


FIG. 4. Change of genotype frequencies over time in one of the five self-incompatible mating types (a), and the linear dominance index (*LDI*) of the population (b) in the site-specific nuclease model, when the recombination rate between the mating type and suppressor loci is greater than the threshold. The parameters and the initial frequencies are the same as in Figure 3 except that the recombination rate is 10-fold greater ($r = 0.05$). The population converges to the isoplethy equilibrium, in which, as in Figure 3, most of the 62 suppressor alleles (differing in their recognition sequences) in the initial population go extinct, leaving only five suppressor alleles with recognition sequences 1, 10, 010, 0100, 10100. These suppressor alleles are maintained at the same frequency in each mating type (a). The genotype frequencies in the other mating types similarly change. As a result, the linear dominance index converges to zero at the equilibrium (b). Thus, there is no dominance relationship between the mating types at equilibrium, though 4/5 of random matings lead to uniparental mitochondrial transmission (the remaining 1/5 are biparental). For example, if two gametes carry the suppressor alleles 10 and 0100, the mitochondria are transmitted only from the 10-carrying gamete, but the direction of transmission is independent of their mating types.

types in the site-specific nuclease model (Fig. 7). The genetic load due to the cost of heteroplasmy or the mutual destruction of mitochondria is minimized under the condition of linear dominance between mating types. This is realized by the buildup of the linkage disequilibrium between the mating-type locus and the suppressor locus, and by the inclusive (nested) subsequence structure in the recognition sequences

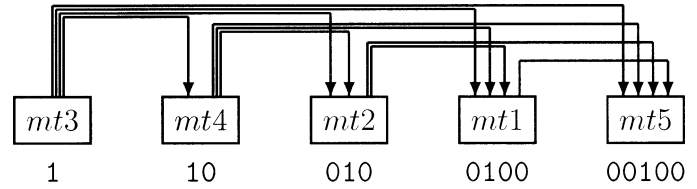


FIG. 5. The final mitochondrial transmission flow (arrows) between five self-incompatible mating types (boxes) at a stable equilibrium of the site-specific nuclease model (Fig. 3). The sequence beneath each box represents the recognition sequence of the suppressor allele that is fixed in the mating type.

of suppressors (Fig. 7). However, we should note that the same load reduction can be achieved in any system in which mitochondrial transmission is uniparental between each pair of mating types. Linear dominance hierarchy is only one such relationship between mating types. Suppose, for example, that there are n mating types in the population. The load is minimized whenever the mitochondrial transmission is uniparental for any pair of mating types. There are $2^{n(n-1)/2}$ different ways of assigning donor-recipient relationship to self-incompatible mating types. The linear dominance hierarchies (with $n!$ different permutations of the order of mating types) are only a tiny fraction (when n is large) of such relationships. Indeed, the fraction

$$R = \frac{n!}{2^{n(n-1)/2}} \quad (12)$$

rapidly approaches zero as n increases. Hence, if arbitrary donor-recipient relationship between mating types is allowed, the linear dominance hierarchy is an exception rather than the rule, in the evolutionary outcomes. Indeed, if n is as large as 15 (as at the *Physarum matA* locus), the fraction of the linear hierarchies in all feasible relationships is $R = n!/2^{n(n-1)/2} \sim 10^{-20}$.

Why, then, is the evolution of the linear dominance hierarchy a robust outcome in our site-specific nuclease model? The key explanation is that the nuclease-methylase scenario mechanistically excludes any cyclic dominance between three or more mating types. The transitive rule (if $A < B$ and $B < C$ then $A < C$) for the order of the mitochondria donor-recipient relationship is naturally incorporated by cut-at-recognition sites and protect-by-methylation mechanisms. Without these mechanisms the linear dominance relationship observed in the mating types of *P. polycephalum* will not evolve and be maintained.

DISCUSSION

We have shown that recombination between mating-type loci and suppressor loci can drastically change the evolutionary outcome for a linear dominance hierarchy of cytoplasmic inheritance. In both the suppression-power model and the site-specific nuclease model, the linkage must be sufficiently tight to realize the buildup of linkage disequilibrium between the mating types and the suppressor loci. If the recombination rate exceeds the threshold, all suppressor alleles are equally distributed among mating types. The

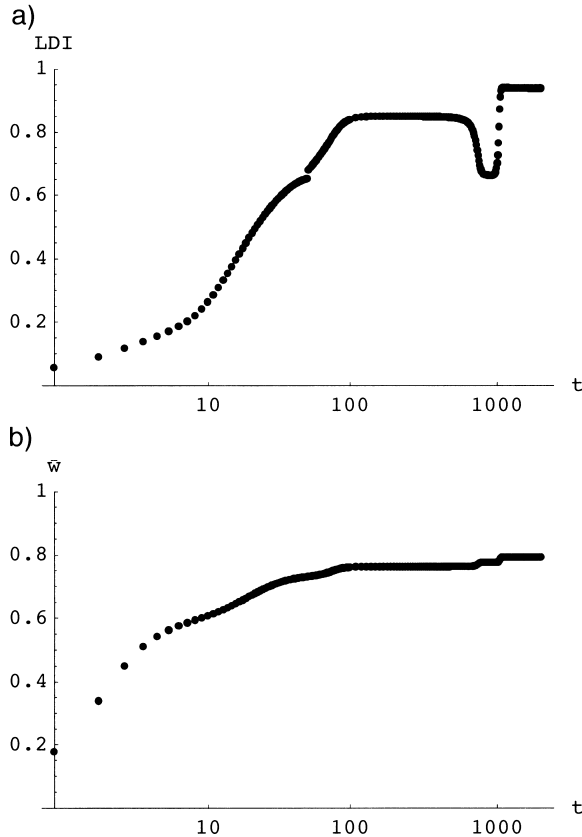


FIG. 6. Change in an index of linear dominance hierarchy (LDI) over time and the mean fitness \bar{w} along the same trajectory as in Figure 3 of the site-specific nuclease model with five self-incompatible mating types. The linear dominance index rapidly approaches one though it stays for a long time at a positive distance from one in this particular run during the period in which mating-types 1 and 4 share the identical suppressor allele 10 (see Fig. 3). During this period the mitochondrial transmission is biparental for the mating between M_1 and M_4 . Finally, around generation 1000, suppressor allele 10 in mating-type M_1 is replaced by suppressor allele 0100, realizing a nearly perfect linear dominance hierarchy ($LDI \approx 1$). The residual deviation is due to the gene flow of suppressor alleles between mating types by recombination. The mean fitness also rapidly approaches its maximum, 0.8, with time. The maximum population mean fitness is $4/5$ because $1/5$ of randomly chosen pairs of gametes are of self-incompatible mating types under the equal frequencies of five mating types.

threshold recombination rate is inversely proportional to the squared number of mating types n , because if n is large, an organism can spread the risk of heteroplasmy by randomizing suppressor alleles. Indeed, the chance that the same suppressor alleles meet in random mating is proportional to $1/n^2$ when all suppressor alleles are equally distributed. Thus, the recombination rate between the mating type and suppressor loci must become disproportionately smaller for the evolution of dominance hierarchy in cytoplasmic inheritance as the number of mating types increases. At the *Physarum matA* locus, where at least 15 mating-type alleles are known, the mating-type locus and the suppressor locus are perfectly linked (both functions are coded by *matA* locus).

Based on the mathematical model, we propose a potential mechanism by which linear dominance hierarchy for

cytoplasmic inheritance observed in *Physarum* mating types evolves and is stably maintained. Our site-specific nuclease model reveals that, as the consequence of the evolution of recognition sequences for nuclease that digests the unprotected mtDNAs, a set of suppressor alleles whose recognition sequences form a nested subsequence relationship is selected for, which is followed by the buildup of nonrandom association of the mating types and the suppressors, thereby realizing a linear dominance hierarchy between mating types for the donor-recipient relationship of cytoplasmic inheritance. The key characteristic of this nuclease-methylase scenario is that it mechanistically excludes any cyclic dominance relationship between three or more mating types. The transitive rule (if $A < B$ and $B < C$ then $A < C$) for the order in mitochondria donor-recipient relationships is naturally incorporated by cut-at-recognition sites and protect-by-methylation mechanisms. The linear dominance is a robust outcome under this recognition-sequence based transitive rule, but is only a negligible exception in the constraint-free suppression network models, suggesting that a nested subsequence structure must play a key role in the *Physarum* system.

Though no information is available as to whether all of the experimentally specified 15 *matA* alleles are segregating in a local population of *Physarum*, our theoretical study suggests that it would be possible. This is because these mating types are subject to a strong balancing selection favoring rare types, and it is well known (e.g., in self-incompatibility alleles in plants and major histocompatibility complex alleles in mammals) that under such selective force a small finite population can maintain a large number of alleles (Wright 1939; Kimura and Crow 1964; Yokoyama and Nei 1979). In an extreme case, at least 37 self-incompatible alleles are found in the population of *Oenothera organensis* with only 500 individuals (Emerson 1938, 1939; Ewens 1964). A great number of mating types observed in basidiomycete fungus *Coprinus cinereus* are determined by the *A* mating-type locus, which is made up of a number of subloci. Several variable regions in the *A* mating-type locus are found to segregate in a natural population and are in linkage equilibrium, thereby ensuring an enormous degree of combinational diversity of fungus mating types (May and Matzke 1995).

Though we started our analysis by assuming that there are already a number of alleles at either mating-type and suppressor locus, our conjecture expected from the present model scheme is that the mating-type locus would first be diversified, followed by diversification of suppressor locus. This is because mating-type alleles by themselves are subject to balancing selection due to the advantage for a rare mating type (Wright 1939; Iwasa and Sasaki 1987), but balancing selection pressure on the suppressor locus arises only after polymorphism is established at the mating-type locus. The next evolutionary step would be the buildup of linkage disequilibrium between the mating-type locus and the suppressor locus. In our model, the linear dominance hierarchy system arises as a consequence of the buildup of linkage disequilibrium, and the buildup process requires, as our results indicate, sufficiently tight physical linkage between the two loci. Suppression of recombination has played a key role in the evolution of the mating-type chromosome in *Neurospora*

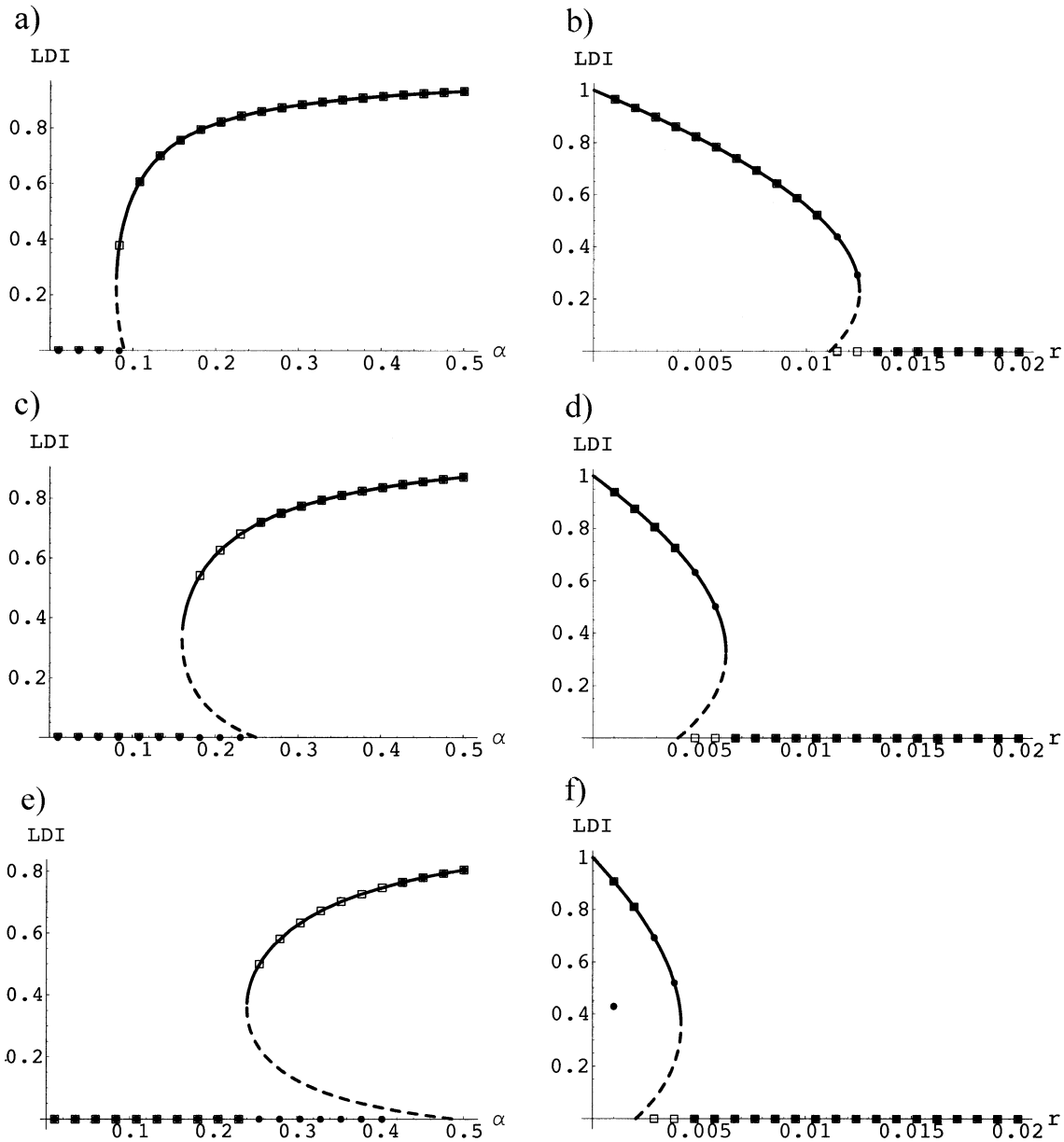


FIG. 7. The bifurcation diagram for the index of linear dominance hierarchy (LDI) plotted against parameters, r the recombination rate and α the degree of heteroplasmic depression, between the mating type and the suppressor loci in the site-specific nuclease model. The solid curve shows LDI at the asymmetrical equilibrium (3) with linkage disequilibrium. The LDI must be zero if the population converges to the isoplethy equilibrium (2). Dots show the LDI observed in the population after 2000 iterations of the genotypic dynamics (7) starting near the asymmetrical equilibrium, and the open squares show those starting near the isoplethy equilibrium. Panels (a), (c), and (e) show LDI plotted against the heteroplasmic depression α (for fixed $r = 0.01$), and panels (b), (d), and (f) show the LDI plotted against the recombination rate r (for fixed $\alpha = 0.1$). The number n of mating types is three in (a) and (b), five in (c) and (d), and seven in (e) and (f).

tetrasperma (Merino et al. 1996), and in the degeneration of the Y chromosome (Rice 1994). Linkage disequilibrium is also thought to play an important role in the functional self-incompatibility in *Brassica*. In *Brassica* species, highly polymorphic self-incompatibility (SI) is controlled genetically by haplotypes consisting of at least two genes, *SLG* and *SRK*. For functional SI to work, these two genes must have shared homology, and the epistatic interaction between them is

thought to be responsible for the linkage disequilibrium and suppressed recombination found between *SLG* and *SRK* (Awadalla and Charlesworth 1999).

In our site-specific nuclease model, the suppressor allele with the greatest specificity in its recognition sequence (e.g., 00100 in Fig. 5) acts identically to the allele that lacks any function of nuclease and methylase function. Though we excluded such a null allele from our analysis, if we include it,

it will replace the position that is occupied by the suppressor allele with the greatest specificity. Thus, in the nested subsequence order relationship established through the dynamics, $s_1 \subset s_2 \subset \dots \subset s_n$, we expect s_n to be a null allele. For the case of $n = 2$, this coincides with the assumption of the Hurst-Hamilton model (Hurst and Hamilton 1992) for the evolution of unidirectional cytoplasmic inheritance and binary mating types (sexes). We have extended their model for a number of mating types far greater than two and revealed that the robust outcome is the linear dominance hierarchy between mating types for the direction of cytoplasmic inheritance, however large the number of mating types. As noted before, this conclusion is robust only under the nuclease-methylase scenario. It is left for future studies if this or a similar molecular mechanism underlies the preferential disappearance of mtDNA in *Physarum*.

The recognition sequence of coexisting suppressor alleles should have a nested subsequence structure (e.g., CGCG \subset TCGCGA). If the linear hierarchy is maintained by the nuclease methylase system, our model predicts that different mating types must share a part of their target sequences for mtDNA digestion.

The linkage between the mating-type locus and the suppressor locus must be sufficiently tight for the evolution of linear dominance. If two loci are loosely linked, linear hierarchy should never evolve or be maintained. The larger the number of mating types, the tighter should be the linkage between mating-type locus and suppressor locus. For example, the recombination rate should be more than 56 times smaller in *Physarum* (where the number of mating types is greater than 15, $n > 15$) than that in *Chlamydomonas* ($n = 2$), if the two systems have the same cost of heteroplasmy.

Different mating types segregate in a natural population at equal frequencies, as is true of the sex-determining alleles and self-incompatibility alleles. Despite the strong rank order relationship among different suppressor alleles, they should be maintained at equal frequencies in the population.

The heteroplasmic depression must be positive in any system with unidirectional organelle inheritance. The heteroplasmic zygote should either have a higher mortality or a reduced growth rate or a higher chance of failing in further development.

The length of the recognition sequence of the suppressor allele (nuclease) must be longer than the number of mating types, and hence must be longer than 15 in *Physarum* (though it can be shorter than 15 if the wobbling rule, as in the relationship between *EcoRII* and *SsoII*, is involved in the nested relationship). Note that recognition sequences of the lengths 20–30 are not exceptional in eukaryotic endonucleases. For example, homing endonuclease *I-CeuI* has the recognition sequence of length 30.

To conclude, our model reveals for the first time that unidirectional organelle transmission can evolve in a system with more than two mating types. Based on the result of our theoretical study, we propose that a nested subsequence structure in the recognition sequence of nucleases that digest organelle DNAs most probably play a key role in the evolution

and maintenance of linear hierarchy in organelle transmission observed in *Physarum*.

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APPENDIX 1

In this appendix, we derive the recursion (1) for the frequency p_{ik} of gamete genotype $M_i S_k$ with the mating-type allele M_i and the suppressor allele S_k . The frequency dynamics are obtained by dividing the life cycle into four steps. First, we consider gamete fusion. Two isogametes are chosen randomly from the gamete pool. If two randomly chosen gametes carry different mating-type alleles, they fuse to form a zygote; if they carry the same mating-type allele, both of them are discarded from the population. The frequency $Z_{ik,jl}$ of a zygote carrying two haploid genotypes $M_i S_k$ and $M_j S_l$ is then

$$Z_{ik,jl} = \begin{cases} p_{ik} p_{jl} / F, & (i \neq j), \\ 0, & (i = j), \end{cases} \quad (\text{A1})$$

with $F = \sum_{i,j} \sum_{k,l} p_{ik} p_{jl} = \sum_{i,j} x_i x_j = 1 - \sum_i x_i^2$, where $\sum_{i,j}$ denotes $\sum_i \sum_{j(\neq i)}$, and $x_i = \sum_k p_{ik}$ is the frequency of mating-type allele M_i .

The second step is viability selection. Zygotes are subject to viability selection due to the conflict between cytoplasm of two parents. If the zygote formed in the first step has the identical suppressor allele, the zygote survivorship is reduced by the amount of α due to heteroplasmic depression. The frequencies of zygote $Z_{ik,jl}^*$ just after the viability selection is

$$Z_{ik,jl}^* = \begin{cases} Z_{ik,jl} / V & (k \neq l) \\ (1 - \alpha) Z_{ik,jk} / V & (k = l), \end{cases} \quad (\text{A2})$$

with $V = \sum_{i,j} \sum_{k,l} Z_{ik,jl} - \alpha \sum_{i,j} \sum_k Z_{ik,jk} = 1 - \alpha \sum_k y_k^2 / F + \alpha \sum_i \sum_k p_{ik}^2 / F$, where $y_k = \sum_i p_{ik}$ is the frequency of suppressor allele S_k .

Next we consider recombination. Two nuclear loci M and S may

recombine during meiosis. The frequency $\tilde{Z}_{ik,jl}$ of the zygote after recombination is

$$\tilde{Z}_{ik,jl} = (1 - r) Z_{ik,jl}^* + r Z_{il,jk}^*, \quad (\text{A3})$$

where r is the recombination rate between the mating type locus and the suppressor locus.

The fourth and final step is gametogenesis. The frequency p'_{ik} of gamete genotype $M_i S_k$ in the next generation is then given by

$$p'_{ik} = \sum_j \sum_l \tilde{Z}_{ik,jl}. \quad (\text{A4})$$

Substituting (A1)–(A3) into (A4) gives

$$\begin{aligned} p'_{ik} &= \frac{(1 - r) \sum_j \sum_l Z_{ik,jl} + r \sum_j \sum_l Z_{il,jl} - \alpha \sum_j Z_{ik,jk}}{V} \\ &= \frac{p_{ik} [(1 - x_i) - \alpha(y_k - p_{ik})] - r(p_{ik} - x_i y_k)}{FV}. \end{aligned} \quad (\text{A5})$$

By defining the gamete fitness by $W_{ik} = 1 - x_i - \alpha(y_k - p_{ik})$, the linkage disequilibrium by $D_{ik} = p_{ik} - x_i y_k$, and the population mean fitness by $\bar{W} = FV$:

$$\bar{W} \equiv FV = 1 - \sum_i x_i^2 - \alpha \sum_k y_k^2 + \alpha \sum_i \sum_k p_{ik}^2, \quad (\text{A6})$$

we obtain the recursion equation for genotype frequencies (1) in the text.

APPENDIX 2

In this appendix we derive asymmetrical equilibria of the suppressor-power model (1), which also gives the frequencies at asymmetric equilibria in the site-specific nuclease model (7)–(8). We assume that there is an equilibrium with strong linkage disequilibrium between the mating type and the suppressor loci, in which each suppressor allele is nonrandomly associated with a different mating type. Without loss of generality we can assume that the suppressor allele i is abundant in the mating-type i . We denote the equilibrium frequency of the abundant suppressor by p (being independent of i) and that of $n - 1$ rare suppressors by q (n is the number of mating types and suppressors):

$$\hat{p}_{ik} = \begin{cases} p & \text{if } i = k \\ q & \text{if } i \neq k, \end{cases} \quad (\text{A7})$$

where $p + (n - 1)q = 1/n$ by definition. From equation (1), we see that the equilibrium frequency \hat{p}_{ik} satisfies

$$\hat{p}_{ik} \hat{W} = [\hat{W}_{ik} \hat{p}_{ik} - r \hat{D}_{ik}]. \quad (\text{A8})$$

Substituting (A7) into this equation yields

$$\begin{aligned} p \hat{W} &= \hat{W}_{ii} p - r \hat{D}_{ii} & \text{if } i = k \\ q \hat{W} &= \hat{W}_{ij} p - r \hat{D}_{ij} & \text{if } i \neq k, \end{aligned} \quad (\text{A9})$$

where \hat{W}_{ii} and \hat{W}_{ij} , defined in (1b), are evaluated as

$$\begin{aligned} \hat{W}_{ii} &= 1 - (1 + \alpha)/n + \alpha p \\ \hat{W}_{ij} &= 1 - (1 + \alpha)/n + \alpha q, \end{aligned} \quad (\text{A10})$$

because $x_i = y_k = p + (n - 1)q = 1/n$. The population mean fitness of gametes is then

$$\begin{aligned} \hat{W} &= \sum_i [\hat{W}_{ii} p + \hat{W}_{ij} q (n - 1)] \\ &= \left[1 - (1 + \alpha) \frac{1}{n} \right] + n\alpha [p^2 + (n - 1)q^2], \end{aligned} \quad (\text{A11})$$

and the linkage disequilibria are:

$$\hat{D}_{ii} = \hat{p}_{ii} - \hat{x}_i \hat{y}_i = p - 1/n, \quad \text{and} \quad (\text{A12a})$$

$$\hat{D}_{ij} = \hat{p}_{ij} - \hat{x}_i \hat{y}_i = q - 1/n. \quad (\text{A12b})$$

We obtain the equations for p and q by substituting (A10)–(A12) into (A9):

$$n\alpha p[p^2 + (n-1)q^2] = \alpha p^2 - r(p^2 - 1/n^2), \quad \text{and} \quad (\text{A13a})$$

$$n\alpha q[p^2 + (n-1)q^2] = \alpha q^2 - r(q^2 - 1/n^2). \quad (\text{A13b})$$

Subtracting (A13b) from (A13a) yields

$$(p-q)\{n\alpha[p^2 + (n-1)q^2] + (r-\alpha)(p+q)\} = 0. \quad (\text{A14})$$

If $p \neq q$, the second factor of the left side of (A14) must vanish. Combining this with $p + (n-1)q = 1/n$, we have a quadratic equation for p (or q), whose roots are

$$p = p_{ii}^{*(\pm)} = \frac{1}{2n} \left[1 \pm \sqrt{1 - \frac{4(n-1)r}{\alpha}} \right],$$

$$(i = 1, 2, \dots, n) \quad \text{and}$$

$$q = p_{ij}^{*(\pm)} = \frac{1}{2n(n-1)} \left[1 \mp \sqrt{1 - \frac{4(n-1)r}{\alpha}} \right],$$

$$(i, j = 1, 2, \dots, n; j \neq i). \quad (\text{A15})$$

There are two branches denoted by + and -, and according to numerical simulations, the (+) branch is locally stable, while the (-) branch is unstable. As the association between mating types and suppressor alleles is arbitrary, the suppressor allele that is abundant in the mating type i can be denoted as k_i , where (k_1, k_2, \dots, k_n) is a permutation of $(1, 2, \dots, n)$. This completes the derivation of (3) in the text.

APPENDIX 3

Here, we derive the conditions under which the equilibrium of model 1 with all gamete genotypes in equal frequencies is stable.

We consider n alleles at both mating-type locus and suppressor locus. Both are assumed to be nuclear loci. The frequency of gamete genotype with mating-type M_i and suppressor allele S_k is denoted by p_{ik} . Letting $x_i = \sum_{k=1}^n p_{ik}$ and $y_k = \sum_{i=1}^n p_{ik}$ be the frequency of i th mating type allele M_i and that of the k th suppressor allele S_k , respectively, the frequency in the next generation p'_{ik} is given as (1) in the text.

We examine the local stability of the isoplethy equilibrium (2), in which all genotypes are segregating with equal frequencies. The equilibrium allele frequencies at each locus are $\hat{x}_i = 1/n$ ($i = 1, 2, \dots, n$) and $\hat{y}_k = 1/n$ ($k = 1, 2, \dots, n$) in the isoplethy equilibrium. To examine the local stability, let q_{ik} be the small deviation of genotype frequency from the equilibrium

$$p_{ik} = 1/n^2 + q_{ik}, \quad (\text{A16a})$$

$$x_i = 1/n + \xi_i, \quad \text{and} \quad (\text{A16b})$$

$$y_k = 1/n + \zeta_k. \quad (\text{A16c})$$

From $\sum_{i=1}^n \sum_{k=1}^n p_{ik} = 1$, we should have $\sum_{i=1}^n \sum_{k=1}^n q_{ik} = 0$. Similarly, $\sum_{i=1}^n \xi_i = 0$ and $\sum_{k=1}^n \zeta_k = 0$.

Substituting (A16) into the recursion equations (1) in the text, and neglecting higher-order terms with respect to q_{ik} , ξ_i , and ζ_k values, we have a linearized system:

$$q'_{ik} = \left(1 + \frac{\alpha - rn^2}{n^2} \right) q_{ik} + \frac{nr - 1}{(n-1)(n-\alpha)} \xi_i + \frac{nr - \alpha}{(n-1)(n-\alpha)} \zeta_k$$

$$\equiv aq_{ik} + b\xi_i + c\zeta_k, \quad (\text{A17a})$$

where

$$a = 1 + \frac{\alpha - rn^2}{n^2}, \quad (\text{A17b})$$

$$b = \frac{nr - 1}{(n-1)(n-\alpha)}, \quad \text{and} \quad (\text{A17c})$$

$$c = \frac{nr - \alpha}{(n-1)(n-\alpha)}. \quad (\text{A17d})$$

The linearized system (A17) can be expressed in a matrix form:

$$\mathbf{q}' = \mathbf{J}\mathbf{q}. \quad (\text{A18})$$

where \mathbf{q} is a n^2 dimensional vector, $\mathbf{q} = (q_{11}, q_{12}, \dots, q_{1n}, \dots, q_{n1}, \dots, q_{nn})^T$, and \mathbf{J} is an $n^2 \times n^2$ matrix, which can be expressed as an $n \times n$ block matrix:

$$\mathbf{J} = \begin{bmatrix} U & V & \cdots & V \\ V & \ddots & \ddots & \vdots \\ \vdots & \ddots & \ddots & V \\ V & \cdots & V & U \end{bmatrix}, \quad (\text{A19})$$

where U and V are $n \times n$ matrices defined as

$$U = \begin{bmatrix} a+b+c & b & \cdots & b \\ b & \ddots & \ddots & \vdots \\ \vdots & \ddots & \ddots & b \\ b & \cdots & b & a+b+c \end{bmatrix} \quad \text{and} \quad (\text{A20a})$$

$$V = \begin{bmatrix} c & 0 & \cdots & 0 \\ 0 & \ddots & \ddots & \vdots \\ \vdots & \ddots & \ddots & 0 \\ 0 & \cdots & 0 & c \end{bmatrix}. \quad (\text{A20b})$$

We now obtain the eigenvalues of \mathbf{J} . The characteristic equation for \mathbf{J} is

$$|\mathbf{J} - \lambda \mathbf{I}'| = \begin{vmatrix} U - \lambda \mathbf{I} & V & \cdots & V \\ V & \ddots & \ddots & \vdots \\ \vdots & \ddots & \ddots & V \\ V & \cdots & V & U - \lambda \mathbf{I} \end{vmatrix}$$

$$= |U + (n-1)V - \lambda \mathbf{I}| |U - V - \lambda \mathbf{I}|^{n-1}, \quad (\text{A21})$$

where \mathbf{I}' is an $n^2 \times n^2$ identity matrix, and \mathbf{I} is an $n \times n$ identity matrix. The determinants in the right side of (A21) are respectively expanded, using the same triangularization technique used in deriving (A21), as

$$|U + (n-1)V - \lambda \mathbf{I}| = (a + nb + nc - \lambda)(a + nc - \lambda)^{n-1}, \quad (\text{A22})$$

and

$$|U - V - \lambda \mathbf{I}| = (a + nb - \lambda)(a - \lambda)^{n-1}. \quad (\text{A23})$$

Thus, the characteristic equation for the matrix \mathbf{J} is

$$|\mathbf{J} - \lambda \mathbf{I}'| = (a + nb + nc - \lambda)(a + nc - \lambda)^{n-1} \times (a + nb - \lambda)^{n-1}(a - \lambda)^{(n-1)^2}. \quad (\text{A24})$$

The associated eigenvector of the eigenvalue $\lambda_1 = a + nb + nc$ is $\mathbf{u} = (1, 1, \dots, 1)^T$ (verified by showing $\mathbf{J}\mathbf{u} = \lambda_1\mathbf{u}$), and hence λ_1 does not affect the dynamics for \mathbf{q} that are restricted in hyperspace $\sum_{i=1}^n \sum_{k=1}^n q_{ik} = 0$. The other eigenvalues determine the local stability of the genetic dynamics are:

$$\lambda_2 = a + nb = \frac{n-2}{n-1}, \quad (\text{multiplicity } n-1), \quad (\text{A25a})$$

$$\lambda_3 = a + nc = \frac{n-2\alpha}{n-\alpha}, \quad (\text{multiplicity } n-1), \quad \text{and} \quad (\text{A25b})$$

$$\lambda_4 = a = 1 + \frac{\alpha - rn^2}{(n-1)(n-\alpha)}, \quad (\text{multiplicity } [n-1]^2). \quad (\text{A25c})$$

Because $|\lambda_2| < 1$ and $|\lambda_3| < 1$ for all n and α assumed in the model ($n \geq 2$; $0 < \alpha \leq 1$), the local stability of the isoplethy equilibrium is determined whether or not the modulus of λ_4 is smaller than one. The destabilization of the isoplethy equilibrium therefore occurs when $\alpha > rn^2$, from which the threshold recombination rate is obtained as $r_c^{(1)} = \alpha/n^2$.

We see that the eigenvalue λ_2 is associated with the perturbation dynamics for the mating-type allele frequencies near the equilibrium. This is shown by (A17):

$$\xi'_i = \sum_{k=1}^n q'_{ik} = \sum_{k=1}^n (aq_{ik} + b\xi_i + c\zeta_k) = (a + nb)\xi_i = \lambda_2\xi_i, \quad (\text{A26})$$

where we used $\sum_{k=1}^n \zeta_k = 0$. Similarly, the eigenvalue λ_3 is associated with the perturbation dynamics for the suppressor allele frequencies near the equilibrium:

$$\zeta'_k = \sum_{i=1}^n q'_{ik} = \sum_{i=1}^n (aq_{ik} + b\xi_i + c\zeta_k) = (a + nc)\zeta_k = \lambda_3\zeta_k. \quad (\text{A27})$$

Finally the eigenvalue λ_4 is associated with the dynamics of the linkage disequilibrium $d_{ik} = q_{ik} - (\xi_i + \zeta_k)/n$ near the equilibrium:

$$\begin{aligned} d'_{ik} &= q'_{ik} - \frac{\xi'_i + \zeta'_k}{n} = aq_{ik}b\xi_i + c\zeta_k - \frac{a + nb}{n}\xi_i - \frac{a + nc}{n}\zeta_k \\ &= a\left(q_{ik} - \frac{\xi_i + \zeta_k}{n}\right) = ad_{ik} = \lambda_4d_{ik}. \end{aligned} \quad (\text{A28})$$

Thus, it is the linkage disequilibrium that is destabilized when r passes downward through the threshold $r_c^{(1)}$.