Dynamics of Plant Mitochondrial Genome: Model of a Three-Level Selection Process

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Manuscript received October 2, 1995
Accepted for publication May 17, 1996

ABSTRACT

The plant mitochondrial genome is composed of a set of molecules of various sizes that generate each other through recombination between repeated sequences. Molecular observations indicate that these different molecules are present in an equilibrium state. Different compositions of molecules have been observed within species. Recombination could produce deleted molecules with a high replication rate but bearing little useful information for the cell (such as "petite" mutants in yeast). In this paper, we use a multilevel model to examine selection among rapidly replicating incomplete molecules and relatively slowly replicating complete molecules. Our model simulates the evolution of mitochondrial information through a three-level selection process including intermolecular, intermitochondrial, and intercellular selection. The model demonstrates that maintenance of the mitochondrial genome can result from multilevel selection, but maintenance is difficult to explain without the existence of selection at the intermitochondrial level. This study shows that compartmentation into mitochondria is useful for maintenance of the mitochondrial information. Our examination of evolutionary equilibria shows that different equilibria (with different combinations of molecules) can be obtained when recombination rates are lower than a threshold value. This may be interpreted as a drift-mutation balance.

THE natural selection of mitochondrial (mt) genomes in plants can be considered as a three-level process: intermolecular, intermitochondrial (these two levels are called intracellular, BIRKY 1994) and intercellular selection. In this paper, we explore with a simulation model the interplay of intercellular, intermitochondrial and intermolecular selection in maintaining or not the entire mt information, and we study the change of proportion of mtDNA molecules within a cell (Figure 1).

The plant mtDNA is composed of a set of different molecules. Restriction maps of cosmid libraries are almost always circular, but observations under the microscope reveal mainly linear molecules (BENDICH 1985; QUÉTIER *et al.* 1985; BENDICH and SMITH 1990). Plant mt genomes are 10- to 100-fold larger than those in animals. The circular genetic map has led to the postulation of the existence of master circles, defined as hypothetical molecules containing the whole mt information. Master circles contain coding, noncoding and recombining sequences. They bear long repeats that do not recombine and short repeats that recombine at specific sites (ANDRE *et al.* 1992). Recombining repeats can be in direct or indirect orientations, the direct repeats being more frequently observed (see HANSON and FOLKERTS 1992 for a review). When two sequences recombine, the initial molecule gives rise to other molecules. Two smaller molecules are produced by recombination through direct recombining repeats, whereas recombination through indirect recombining sequences gives rise to one molecule of the same size but with a modification of sequence orientation (QUÉTIER *et al.* 1985). Recombination between recombining sequences generates a population of molecules (LONSDALE *et al.* 1988) of different sizes and molecular structures (an example in Figure 2).

Experimental data have shown that mtDNA molecules can be in different proportions within the same species. For example, different mitochondrial cytotypes corresponding to various compositions of molecules have been observed in *Zea mays* (SMALL *et al.* 1987). Some molecules may be substoichiometric (very low frequencies) in a cytotype and frequent in another one. The amplification of preexisting substoichiometric molecules as a mechanism of variation of the mt genome was suggested for the first time by SMALL *et al.* (1987). Such a process of amplification of substoichiometric molecules would permit rapid evolution of the mt genome structure and explain why great variability is found in the gene order between closely related species

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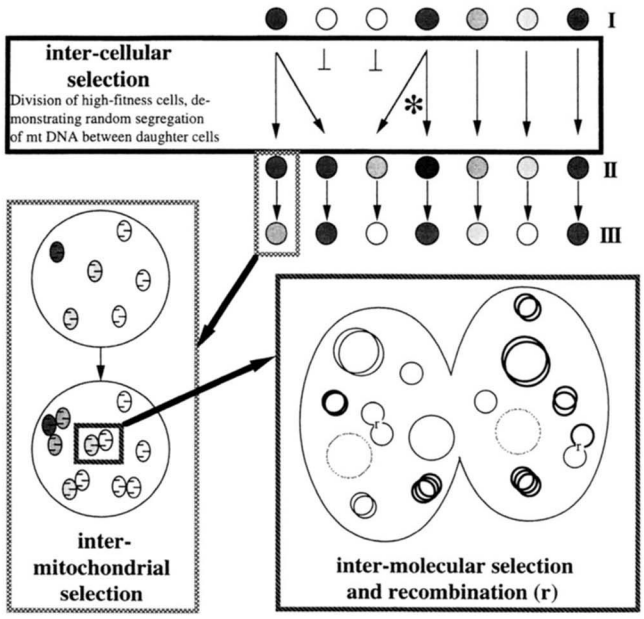


FIGURE 1.—Model of mitochondrial evolution with three levels of selection. The darker the pattern, the higher the fitness (for cells, mitochondria or molecules). The highest level of selection considered here, namely intercellular selection (represented at the top of the figure), consists of the differential survival and replication of "high-fitness" cells (dark gray) relative to "low-fitness" cells (light gray) (the method of determining the relative fitness of cells is described below). During cell division (represented in the frame entitled "intercellular selection"), mtDNA randomly segregates between daughter cells, leading in some cases to inequality between the cells (*). Before the next cell division, mtDNA replicates within mitochondria (cross-hatched frame). If intermitochondrial selection occurs, mtDNA replication differs between relatively high-fitness (dark gray) and low-fitness (light gray) mitochondria (the method of determining relative fitness of mitochondria is described below). Within each mitochondrion (hatched frame) DNA molecules replicate according to the number of replication origins on the molecule. Selfish molecules (bold circles), with a high density of replication origins, replicate relatively fast; while molecules with no replication origins (dashed circles) do not replicate at all. In addition, mtDNA can recombine (indicated by an *r*). Mitochondrial fitness is determined by the composition of all mtDNA sequences within the mitochondrion. A mitochondrion principally composed of selfish sequences of mtDNA (*e.g.*, the mitochondrion on the right in the magnified view, appearing light gray in the unmagnified view) has relatively low fitness, whereas a mitochondrion principally composed of sequences containing a sufficient amount of information has relatively high fitness (the dark gray mitochondrion in the unmagnified view). Some genetic complementation between mitochondria is possible (see text for additional details). A cell's fitness is the sum of the fitnesses of the mitochondria it contains. As a result of intercellular selection, mean fitness increases (*e.g.*, the lower line of cells is darker than the upper one). In contrast, intracellular selection (intermolecular selection compensated to some extent by intermitochondrial selection) decreases mean cell fitness (*e.g.*, the third line of cells is less dark than the second line). The resulting system, with three levels of selection, can reach an equilibrium at which alternating generations of cells have an equal mean fitness.

or within the same species (PALMER and HERBON 1988). In contrast, little variability has been found in DNA sequences, indicating that the evolution of nucleotide sequences is very slow. The substitution rate is of the order of 0.2 substitution per site per billion years, which is 10 times less than in the nucleus and hundred times less than in animal mitochondria (WOLFE *et al.* 1987). Therefore, the evolution of the plant mitochondrial genome seems to proceed more by rearrangement than by nucleotide substitution. *In vitro* culture is the only experimental system so far where a change of equilibrium state of mt molecules has been observed directly (HANSON and FOLKERTS 1992; VITART *et al.* 1992).

This particular mode of evolution was theoretically modelled by ATLAN and COUVET (1993) to determine what types of equilibria of molecules are possible. Their simulation model involves an intermolecular level of selection: molecules of various sizes and structure replicate differentially. Several dynamic equilibria of molecules can exist according to the recombination rate values. The model shows that these dynamics alone lead to an invasion of molecules bearing little information. This results either from differential reproduction in favor of small molecules or drift. In the absence of a specific force maintaining it, most of the information is lost. Indeed, a master circle having, for example, one replication origin should replicate in a biological organism more slowly than a small circle with one replication origin. This is another example of conflict linked with the cellular and mitochondrial levels (COSMIDES and TOOBY 1981). In ATLAN and COUVET's model, the whole mt information is maintained if a replicative advantage is given to the master circle. Indeed, other molecules seem to have a potential for faster replication than master circles, either because they have the same number of replication origins but are smaller than master circles, or because they are of similar sizes but have more numerous replication origins. Unfortunately for the cells that bear them, these molecules carry little information, since sequences coding for mt functions are replaced by sequences favoring replication. Such molecules will be refered to "selfish circles" (DOOLITTLE and SAPIENZA 1980; ORGEL and CRICK 1980).

Another process able to preserve the mt information (*i.e.*, preventing invasion by selfish molecules) without a replicative advantage of master circles could be an intercellular and/or an intermitochondrial selection process. Intercellular selection prevents cells without enough information from dividing. One can expect that molecules in a mitochondrion with all mt information replicate better than molecules in a deficient mitochondrion. One would expect therefore that the intercellular and intermitochondrial level of selection counteract the intermolecular level: they favor the presence of information whereas intermolecular selection favors selfish molecules.

Our model was run with and without a mitochondrial level of selection. Indeed the fusion of mitochondria into a single chondriome has been postulated but has not been clearly observed experimentally. Microscopy pictures generally show isolated mitochondria (BENDICH and GAURILOFF 1984). However, in some hybrids obtained by protoplast fusion, recombination rapidly occurs between the two mitochondrial genomes, indicating that mitochondria fuse (BELLIARD *et al.* 1979). In the presence of a chondriome, there is no spatial structure and thus no selection at the mitochondrial level. However if sometime in the cell cycle, mitochondria are isolated and develop separately from each other, selection at the mitochondrial level can occur.

A number of papers have already described intracellular selection (*e.g.*, BIRKY 1973; TAKAHATA and SLATKIN 1983; BRENDEL and SEGEL 1987; CONDIT and LEVIN 1990). The aim of this paper is to introduce intermitochondrial and intercellular selection in a model of plant mt genome dynamics, and to use this model to investigate mt evolution in plants. The first questions concern the maintenance of mt information. How is the entire mitochondrial information maintained? Can intercellular selection alone explain the maintenance of the entire mt information? If not, do intercellular and intermitochondrial selection together maintain it? What types of molecules are responsible for the maintenance of information? The second set of questions concerns the composition of the mitochondrial genome. Is it possible to get several equilibria of mt molecules? What are the main characteristics of these equilibria? And how can the switch from one equilibrium to another one occur? The understanding of the different equilibria of mt molecules and the change of equilibrium should provide information on evolutionary process of the plant mt genome.

THE MODEL

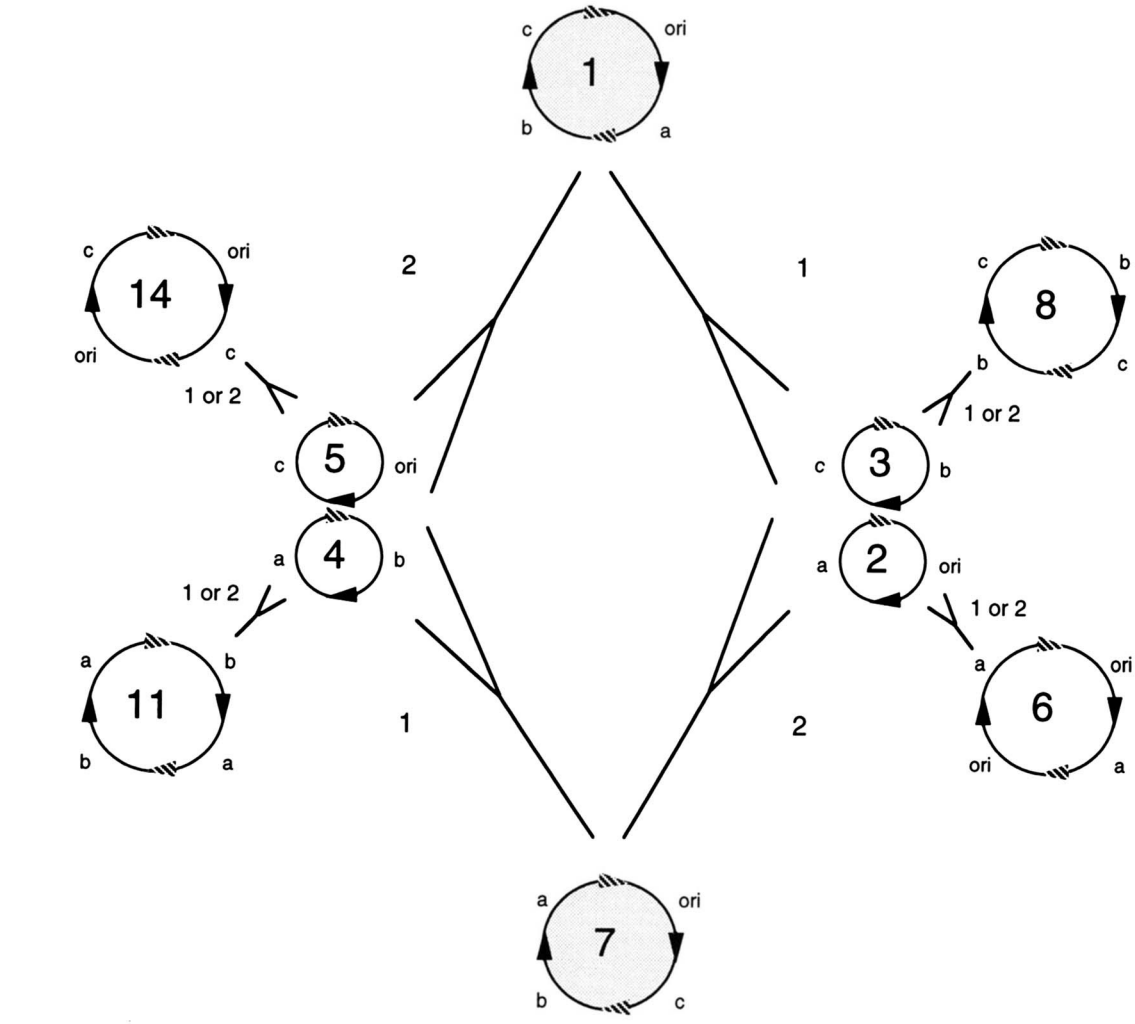
To simulate the evolution of plant mtDNA molecules within a cell population, a theoretical model was constructed that takes into account the main mechanisms of mt genome evolution. As in ATLAN and COUVET (1993), the evolutionary forces, namely recombination, selection of molecules as a result of their differential replication and drift resulting from random segregation of molecules during cellular division, are assumed to act successively during each cell cycle. A cell cycle begins with the recombination process; after mt molecules are apportioned among the mitochondria, replication takes place and mt molecules are randomly distributed between the two daughter cells. In our model, the selection is acting at three different levels: intermolecular, intermitochondrial, and intercellular. The model is a simulation model written in turbo pascal for a PC with a pentium processor. The program can be obtained by contacting the authors. Each set of parameters were run 40 times.

Main assumptions of the model: The set of molecules on which selection acts is generated through recombination of recombining repeats. This can happen either between two repeats of the same circle or between two repeats of two different circles. The possible molecules depend on the number, position, and orientation of the recombining sequences. We assume in the model that recombination rates between recombining sequences depend on the type of recombination (between or within molecules), and that a molecule can recombine only once per cellular cycle. However, if two different molecules with the same recombining sequences are always able to fuse, the number of polymers generated by the fusion process is potentially infinite. Experimental observations do not permit to determine clearly the size of mt molecules; therefore, in the model, we limited the maximum size of molecules to that of the master circle.

Intermolecular selection favors molecules with little information but with a lot of replication origins. This was modelled considering that a molecule in a given mitochondrion will replicate faster if its replication origin density increases (the ratio of the number of replication origins over the length). But since the resources for mtDNA replication in the cells are limited (suggested by BIRKY 1994), they must be split between the replicating molecules, so that a molecule replicates less frequently as the number of replication origins in the cell increases.

Intercellular and intermitochondrial selections favor cells and mitochondria that contain all functional sequences in sufficient amounts (see below). Through intercellular selection, cells with complete information and enough sequences of each type will divide, whereas cells with insufficient information will not. In the same way, through intermitochondrial selection, molecules in mitochondria with more information will replicate more than molecules in mitochondria with little information. Furthermore, we consider that a deficiency of one type of region containing different sequences within a mitochondrion can be compensated within a cell containing a large amount of this region. This is assuming complementation between mitochondria of the same cell (suggested by OLIVER and WALLACE 1992).

Intracellular variability and the population of circles: We simulated circular molecules that are a more general case than linear molecules. We chose a system of circles with four regions of equal length, three of them containing coding sequences and the other one containing the origin of replication and no other significant information. A master circle with two direct recombining sequences (circle 1 or 7 in Figure 2) was chosen. Such a circle generates a system with a reasonable number (14) of circles (Figure 2). Master circles (circles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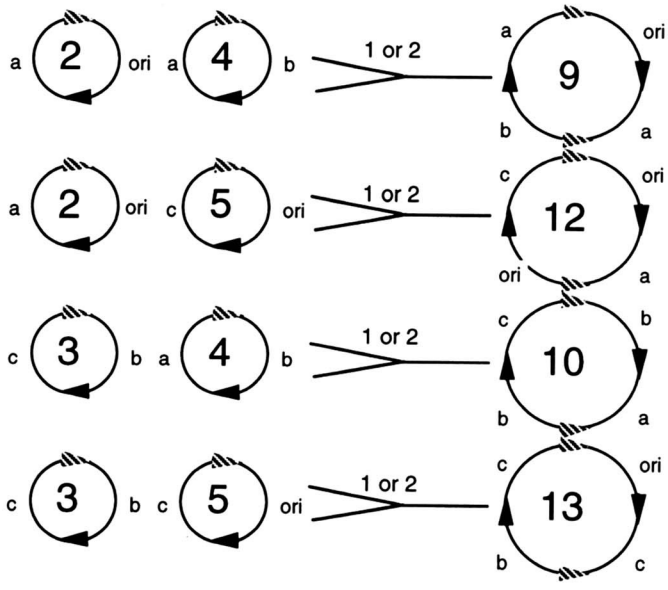
2
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13

1 and 7 in Figure 2) contain two direct recombining sequences and four different regions: a region with the replication origin and three others with coding sequences containing together all the mt information. There are two master circles differing by the orientation of the coding sequences. Four small circles (two regions each, circles 2–5 in Figure 2) are generated by intramolecular recombination within master circles. Only two small circles (circles 2 and 5 in Figure 1) bear the region with the replication origin. They are called small selfish circles because they replicate a lot (small size) and bear only one region with coding sequences useful for the mitochondrion. Three circles (circles 6, 12, 14 in Figure 2) have two regions with the replication origin and two other regions; they are called large selfish circles. Three others have no replication origin (circles 8, 10, 11 in Figure 2) and four regions with coding sequences. The last two circles (circles 9 and 13) have one origin of replication and three coding sequences, one of which is found twice. These two circles together possess the same information as two master circles.

Recombination process: A molecule recombines at different rates depending on the type of recombination process (intra- or intermolecular recombination). It is assumed that recombination among molecules occurs randomly between all molecules in a cell (panmictic population of molecules), as suggested by LONSDALE *et al.* (1988). We assume that a circle can recombine only once per generation.

For simplicity, the simulations were realized using a system of circles with only direct recombining sequences. The rate of intramolecular recombination is called R_{intra}. R_{intra} is the probability for a molecule to be choosen to recombine and if the molecule can undergo intramolecular recombination, recombination is completed. The probability of meeting and recombining between two molecules is called R_{inter}. When recombination occurs, it takes place at random between all the possibilities. With the system of circles used, two circles share always at least one recombining sequence.

Intermolecular selection: One of the assumptions of the model is that within a given mitochondrion, some molecules replicate more than others because of the high density of replication origins that they carry. In the system used in this study, there are only four types of circles: circles that do not replicate (no replication origin), circles with one region with the origin and three regions with coding sequences (among which are the two master circles), circles with one region with origin and one region with coding sequences (called small selfish molecules), and large circles with two origins (large selfish circles). Therefore, there is a difference in the density of replication origins between selfish circles and large circles with just one replication origin when they replicate within the same mitochondrion. The fitness of selfish molecules relative to molecules having one origin of replication for three regions with coding sequences is assumed to be r, with $r \geq 1$. The higher the value of r, the stronger the molecular selection. Therefore, the fitness of a molecule, $W_{molecule}$ can be expressed as:

$$\begin{aligned} W_{molecule} &= 0 \quad \text{if the molecule has no replication origin (circles 8, 10, and 11 in Figure 2),} \\ W_{molecule} &= 1 \quad \text{if the molecule has three regions with coding sequences and one origin (master circles 1 and 7),} \\ W_{molecule} &= r \quad \text{if the molecule is selfish (circles 2, 5, 6, 12 and 14).} \end{aligned} \tag{1}$$

Intercellular and intermitochondrial selection: Intercellular selection was modelled in two ways: either a truncated or a probabilistic selection. In the truncated cellular selection, it is assumed that only a fixed number x of best cells (higher fitness) divide and the same number of worst cells are eliminated. In the probabilistic cellular selection, the probability for a cell to divide corresponds to its relative cellular fitness, x cells are chosen. The same number x of cells is chosen to die with a probability corresponding to its relative cellular fitness. In both cases, a cell is composed of a set of mitochondria, and the cell population is constant (50 cells). Molecules are randomly distributed between the two daughter cells (BIRKY 1983). A good cell [high cellular fitness (W_{cell})] possesses good mitochondria [high mitochondrial fitness (W_{mito})]. This can be simulated using the following equation:

$$W_{cell} = \sum_j W_{mito}(j). \tag{2}$$

FIGURE 2.—Population of mitochondrial molecules. A mitochondrial genome containing several regions was chosen. The different regions are as follows: ori, a, b, c, 1 and 2. ori contains the replication origin and other sequences not useful; a, b, c are long regions (containing coding and no coding sequences), and 1 and 2 are direct recombining repeats. The gray arrow represents the recombining sequence 1 and the black one, the recombining sequence 2. The orientation of the sequence is indicated by the direction of the arrow. The master circle 1, whose sequence composition is 1ori2a1b2c generates circles 2–14 through recombination between the recombining sequences. Lines relate circles that generate each other through recombination. The numbers above the line indicate which type of recombining sequence is involved in the recombination. The end of the line indicates whether the recombination event happens within or between molecules (for example, from circles 2 and 3 to 1 the recombination is intermolecular, and from circle 1 to 2 and 3 the recombination is intramolecular). Shaded circles contain complete mitochondrial information, *i.e.*, are master circles. Circles 2 and 5 are called small selfish circles, and circles 14, 12 and 6 are called large selfish circles.

Well-functioning mitochondria require a certain amount of genetic information, which means at least a minimum of all regions. A mitochondrion is assumed to contain 10 circles chosen randomly between all the cell's mt circles (BIRKY 1983). The repartition of the molecules is done after the recombination process and before replication (*i.e.*, the selection). A good balance between all the mt regions is considered necessary to have a good mitochondrion. If one region is missing, the mitochondrion cannot function (no replication). It is possible to obtain such a pattern by considering that the contributions of every region interact multiplicatively to give the mitochondrial fitness. W_{reg} represents the contribution of a region (i) to the fitness of a mitochondrion (j), the mitochondrial fitness (W_{mito}) can be modelled as the product of the contribution of each type of region:

$$W_{mito}(j) = \prod_i W_{reg}(i,j). \quad (3)$$

If some regions are less represented than others, the mitochondrial fitness is decreased. However, the mitochondrion can be complemented by other mitochondria, *i.e.*, the lack of a sequence in a mitochondrion can be compensated for by the presence of this sequence somewhere else in the cell (suggested by OLIVER and WALLACE 1992). Complementation can occur during fusion of mitochondria or maybe by transfer of gene products from one mitochondrion to another (the actual mechanism is not known, but exchanges have been detected). We modelled this phenomenon by considering that the contribution of a region to the mitochondrial fitness is influenced by the overall frequency of this region in the cell. The negative effect of the lack of a region in a mitochondrion is more drastic when this region is rare in the cell. The way we calculated $W_{reg}(i,j)$ allows us to model this effect. $W_{reg}(i,j)$ is maximal (equal to one) if the mitochondrion has its optimum number or more of region i (see Equation 4). If there is less than the optimum number of a region in the mitochondrion, the contribution of the region (W_{reg}) to the mitochondrial fitness decreases (when $N_{opt} - N_{real}(i)$ increases, $W_{reg}(i,j)$ decreases). The contribution decreases the rarer the region is in the cell, *i.e.*, when the frequency within the cell of the region i ($Nc_{reg}(i)$) relative to the commonest region in the cell ($(Nc_{reg}(i))$ decreases, $W_{reg}(i,j)$ decreases, (see Equation 5). Therefore, $W_{reg}(i,j)$, the contribution of the region to the W_{mito} can be calculated as follows:

$$\text{if} \quad N_{real}(i) > N_{opt},$$

$$\text{then} \quad W_{reg}(i,j) = 1, \quad (4)$$

$$\text{else} \quad W_{reg}(i,j) = [Nc_{reg}(i)/Nc_{reg}(k)]^{(N_{opt}-N_{real}(i,j))}, \quad (5)$$

with $Nc_{reg}(i)$ = number of regions i in the cell, N_{opt} = optimum number of regions in the mitochondrion to have a well-functioning unit and $N_{real}(i, j)$ = real number of regions (i) in the mitochondrion (j).

The replication of a molecule within a cell depends on the type of this molecule and the mitochondrion containing this molecule. The ability of a molecule to replicate is expressed by $W_{molecule}$ (explained earlier). The replication takes place inside mitochondria. Mitochondrial selection is expressed by considering that replication of a given type of molecule is proportional to the quality of the mitochondrion (measured by W_{mito}) containing the molecule. Moreover, since cellular resources used for mt replication are limited (suggested by BIRKY 1994) and allocated to each molecule, the more replication origins within a cell, the less the replicating ability conferred by each individual origin. The replicative rate of a molecule ($R_{molecule}$) is therefore supposed to be inversely proportional to the total number of replication origins within a cell. This can be modelled using the following equation:

$$R_{molecule} = W_{mito} * W_{molecule} * k / Nc_{ori}, \quad (6)$$

where W_{mito} represents the ability of a mitochondrion to replicate its molecules (see Equation 3), $W_{molecule}$ represents the ability of a molecule to replicate (see Equation 1), k is a constant expressing the total amount of resources allocated to mitochondrial replication in the cell, and Nc_{ori} is the number of replication origins in the cell. The number of copies produced for a given molecule was computed from a Poisson distribution using the replication rate $R_{molecule}$ as a parameter.

The absence of an intermitochondrial selection level was simulated in the same way but instead of having a mitochondrion with randomly chosen circles, we defined an average mitochondrion. An average mitochondrion is composed of an average frequency of all the sequences present in the cell. W_{mito} and W_{reg} were calculated in the same way.

RESULTS

Two-level model with intermolecular and intercellular selection; maintenance of the mitochondrial information: The maintenance of mt information strongly depends on the strength of intermolecular selection. For values of r (the relative fitness of selfish molecules) greater than a threshold value lying between 1.2 and 1.4, selfish molecules always increase in frequency; the cell fitness decreases at the same time (Figure 3). The cell fitness is close to zero, but there are still some molecules in the cells. The percentage of selfish circles is high, but some master circles and others are still present. When the advantage of selfish circles decreases (r decreases and is close to 1.2–1.4, simulations 1 to 8 Table 1), the information can be maintained (the intermolecular selection pressure decreases).

The threshold value of r to maintain mt information

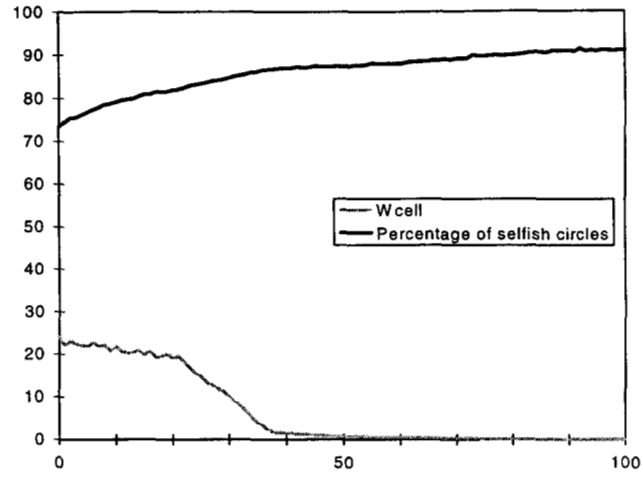

FIGURE 3.—Loss of mt information with two levels of selection. The simulations were done with molecular and cellular selection levels. The percentage of selfish circles (circles 2, 5, 6, 12 and 14) is shown over the generations. The average fitness of the cell population is represented over the generations. The selfish circles increase and W_{cell} decreases at the same time. Finally, there are not enough molecules in the cells, so these go extinct. The simulation parameters are as follows: $r = 2$, $R_{inter} = R_{intra} = 0.1$, $x = 5$, $N_{opt} = 3$, no mt selection and truncated cellular selection.

depends on parameter values (see simulations in Table 1) and on the type of cellular selection. When the optimum number for one type of region in a mitochondrion increases (simulations 1–2), the information is lost more easily. An increase in intermolecular recombination rate leads to an increase in average fitness of the cell population (simulations 2–4). An increase in intramolecular recombination rate gives rise to a decrease in average cellular fitness (simulations 2–3). When the number of cells that divide increases, the equilibrium state of the cellular fitness is reached less rapidly. The threshold value for the maintenance of mt information is always higher under truncating selection than under probabilistic selection (simulations 2–6).

Three-level model, with intermolecular, intermitochondrial and intercellular selection; maintenance of the mitochondrial information: The mt information is always maintained with these three levels of selection. For every simulation, whatever the parameters (we tested values of r up to 2), the average fitness of the cell population reaches an equilibrium with no loss of information. This equilibrium does not depend on the initial composition of circles within the cell population. In Figure 4A, three simulations are shown, starting with three different circle compositions in the cells. The same equilibrium of cell fitness is reached.

Influence of parameters: See the simulations in Table 1. The average fitness of the population of cells can vary up to fivefold according to the parameters. The average fitness of the cell population decreases when there is an increase of the optimum number of regions needed in a mitochondrion to be a well-functioning unit (simulations 9–12). This effect can be explained by the fact that when little information is needed, a mitochondrion is more readily "good" and as a consequence the average fitness of cells is higher. An increase

TABLE 1

Influence of the parameters

Simulation	Mitochondrial selection	Truncated cellular selection	r	R_{inter}	R_{intra}	N_{opt}	x	Average of W_{cell}	Average of W_{mito}	Average no. of circles	Average of the percentage of master circles
1	−	+	1.4	0.1	0.1	3	5	0		150	5
2	−	+	1.4	0.1	0.1	1	5	2.5		460	3
3	−	+	1.4	0.1	0.3	1	5	1.5		260	3
4	−	+	1.4	0.3	0.1	1	5	3		490	3
5	−	+	1.4	0.3	0.3	1	5	2		330	3
6	−	−	1.4	0.1	0.1	1	5	1		190	2
7	−	+	1	0.3	0.3	3	5	5		260	8
8	−	+	1.2	0.3	0.3	3	5	0		150	5
9	+	+	2	0.1	0.1	3	5	18	0.34	560	6
10	+	+	2	0.3	0.1	3	5	18	0.35	520	8
11	+	+	2	0.1	0.3	3	5	10	0.24	450	6
12	+	+	2	0.1	0.1	1	5	47	0.55	870	5
13	+	+	2	0.1	0.1	3	15	18	0.34	560	11
14	+	−	2	0.1	0.1	3	5	10	0.31	350	5

Mt selection is the occurrence or not of a mitochondrial level of selection, the cellular type of selection (truncated or not) is indicated, r represents the importance of intermolecular selection, R_{inter} and R_{intra} are the recombination rates, N_{opt} is the optimum number of a type of sequence in a mitochondrion to get a well-functioning unit, and x is the number of cells that divide per generation. The simulations were run to an equilibrium state of the cellular fitness. The average values given are calculated from a part of the run, *i.e.*, the equilibrium state of the cellular fitness. It is an average of all the cells at a given generation and of the generations at the equilibrium state reached for the cellular fitness.

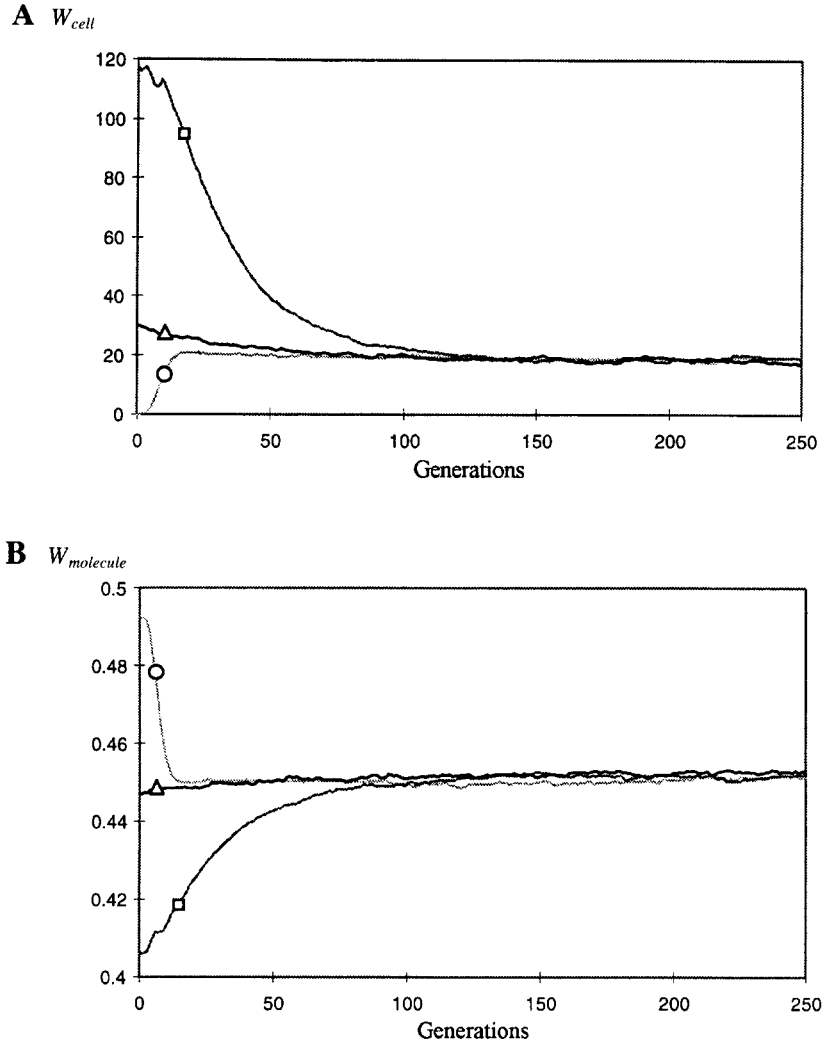

FIGURE 4.—Maintenance of mt information with three levels of selection. The simulations were done with molecular, mitochondrial and cellular levels of selection. (A) The average fitness of the cell population is represented over the generations for three various initial proportions of the different circles. It reaches an equilibrium, with no loss of information, whatever the initial situation. (B) The mean molecular fitness, here equal to the number of replication origins per sequence, is shown over the generations. Intercellular and intermolecular fitnesses stabilize simultaneously. The simulation parameters are as follows: $r = 2$, $R_{inter} = R_{intra} = 0.1$, $x = 5$, $N_{opt} = 3$, mt selection and truncated cellular selection (this simulation correspond to the simulation 9 in Table 1). The initial composition of circles are as follows: the simulation with the square has 447 master circles, 774 small selfish circles, 185 large selfish circles, 50 small circles with no origin of replication; the simulation with the triangle has 127 master circles, 412 small selfish circles, 184 large selfish circles, 14 small circles with no origin of replication; the simulation with the circle has nine master circles, 275 small selfish circles, 36 large selfish circles.

in the number of cells that divide at each generation does not affect the final equilibrium (simulations 9–13), but this equilibrium is reached faster. Probabilistic selection leads to higher values of cell fitnesses variance and to lower values of mean cell fitness (simulations 9–14). Recombination rates also have an effect on cell fitness. Cell fitness decreases with an increase of the intramolecular recombinations (breakdown of molecules, simulation 9–11) and decreases or stays the same with an increase of the intermolecular recombination rate (fusion of molecules, simulations 9–10).

The average fitness of the molecules reaches an equilibrium when the average fitness of the cells stabilizes (Figure 4B). Cellular and molecular fitness stabilize at the same time (Figure 4).

Master circles and mt information maintenance: The total number of molecules within a cell has an important role for cell fitness. The average fitness of the cells is highly correlated with the average number of circles within a cell, whatever the parameters (Spearman correlation coefficient of 0.95, $P < 0.001$). This is calculated from different simulations with various sets of parameters, an average value per simulation is used. The average total number of circles within cells reflects the fitness of cells. Master circles are not essential for maintenance of the mt information. Indeed, even when master circles represent a few percent of the circles present in the cells, the mt information can be maintained (Figure 5). When master circles are nearly absent, circles 9 or 13 are present in high proportions. When the number of sequence needed to get a well-functioning mitochondrion (N_{opt}) is lower, the number of selfish circles increases and the number of master circles decreases.

Equilibria of mitochondrial molecules with two or three levels of selection: An equilibrium of molecules is defined by a constant stoichiometry of the molecules over a large number of generations. Two situations can be observed concerning equilibria of molecules: either there is only one state (Figure 6B) or four different equilibria can be observed (Figures 5, A and B, and 6). In the second case, two out of the four equilibria differ in the proportions of the two types of master circles (circles 1 and 7, Figure 2). One equilibrium state is

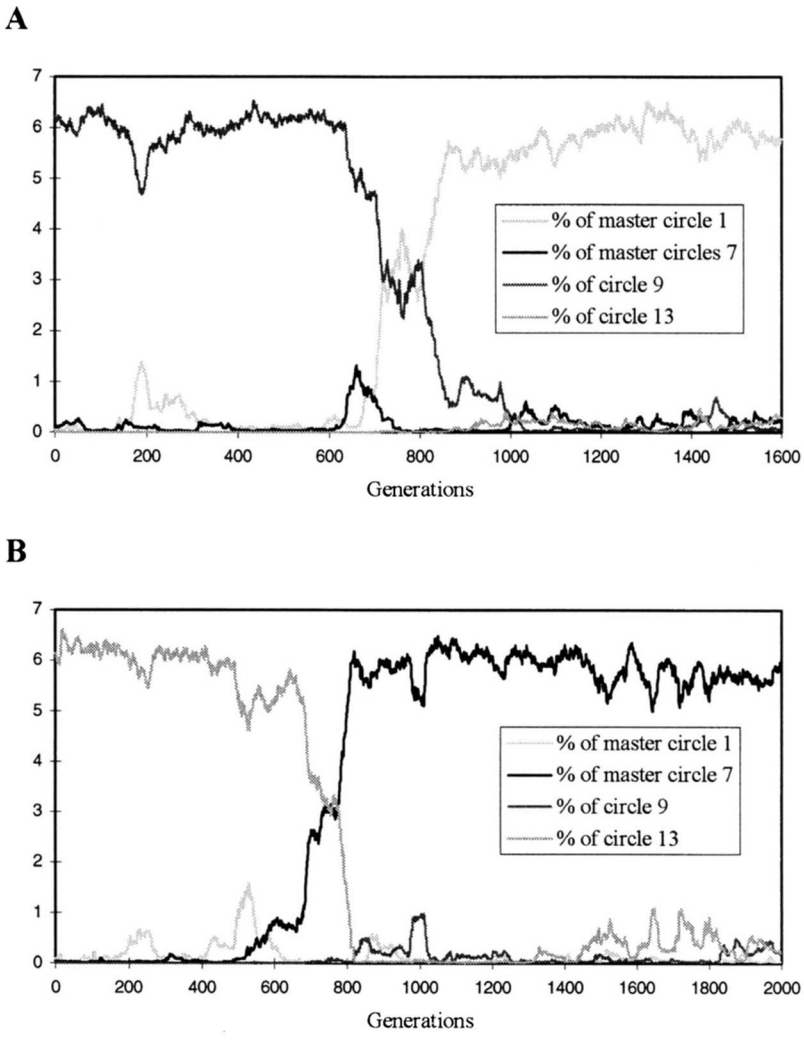

FIGURE 5.—Equilibria of molecules with a low frequency of master circles. The mt information can be maintained with few master circles in the population. The percentage of master circles and the percentage of circles 9 and 13 are represented over the generations. (A) Two equilibrium states are shown. In the first one master circles are abundant and circles 9 not, in the second one circles 9 are abundant and master circles are in low frequency. A transition from these two equilibria is shown. (B) Two equilibrium states are shown. In the first one master circles are abundant and circles 13 not, in the second one circles 13 are abundant and master circles are in low frequency. A transition from these two equilibria is shown. The simulation parameters are as follows: $r = 2$, $R_{inter} = 0.08$, $R_{intra} = 0.08$, $x = 5$, $N_{opt} = 1$, mt selection and truncated cellular selection.

characterized by the abundance of master circle 1 and a low frequency of master circle 7. The second equilibrium state is characterized by a high frequency of master circle 7 and a low frequency of master circle 1 (Figure 6). The third and the fourth equilibria (Figure 5, A and B) are characterized by a low frequency of both master circles and a high frequency of circles 9 or 13. In all cases, the transitions from one equilibrium to another are not accompanied by a decrease of the fitness of the population of cells. The four different types of equilibria are reached randomly, not in a regular pattern.

Influence of the parameters: The frequency of change of equilibrium depends on the recombination rates: the higher the recombination rates, the higher the transition number from one equilibrium to another. When these transitions become very frequent (R_{inter} and R_{intra} close to 0.2), equilibria can no longer be distinguished (Figure 7). There is only a continuity of states, and one can go from one to another with high probabilities (Figure 6B). The type of equilibria do not depend on the type of cellular selection (truncated or probabilistic), the number of cells that divide (x) and of the replicative advantage of selfish molecules (r). The mitochondrial selection (N_{opt}) has an influence over the type of equilibria. That is, if the mitochondrial selection is low, some equilibria between the two master circles and one another (circle 9 or 13) are possible, but if the mitochondrial selection is high, the equilibria of molecules concern only the two master circles. The value of the equilibria depends on the type of cellular selection and on the strength of the mitochondrial selection, but exactly the same equilibria values are obtained for different advantages of selfish molecules (when there are three levels of selection) and for various values of the number of cells that divide.

DISCUSSION

The maintenance of information by multilevel selection: The above results confirm that mt information may be lost and suggest that the mitochondrial level of selection has an important role in maintaining mitochondrial information.

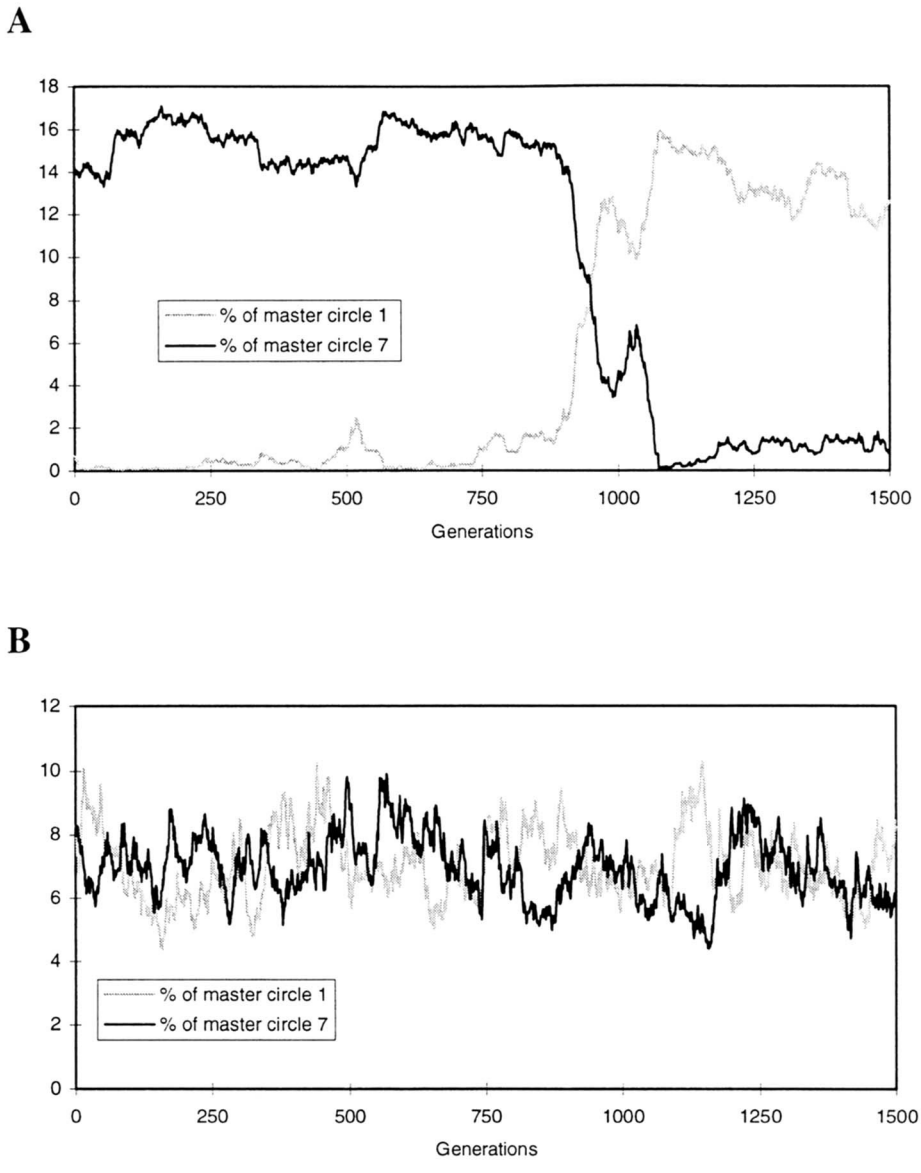

FIGURE 6.—Equilibria of molecules. (A) The average percentage of the two different master circles (circles 1 and 7) of the cell population are represented over the generations for low recombination rates (0.1). Two different equilibrium states are shown with one type of master circle abundant and the other one in low frequency. There is a transition from one equilibrium to the other one. (B) The average percentage of the two master circles are represented over the generations for high recombination rates. There is only one state with equal proportions of both circles. The simulation parameters are as follows: $r = 2$, $R_{inter} = R_{intra} = 0.1$ (A), $R_{inter} = R_{intra} = 0.6$ (B), $x = 5$, $N_{opt} = 3$, mt selection and truncated cellular selection.

In the two-level selection model (with intermolecular and intercellular levels of selection), when there is a significant advantage to selfish circles in intermolecular selection, the average fitness of cells is close to zero. In the model, we have assumed that recombination and replication of molecules occur only once per generation. If they occur more than once, the advantage of selfish circles will probably be higher. When the average fitness of cells is close to zero, cells cannot divide, and thus the population of cells becomes extinct because information in molecules contained in the mitochondria is not sufficient to give a functional cell. Selfish circles invade the cell because the selection at intercellular level cannot compensate for the high advantage given to selfish sequences in intermolecular selection. Nevertheless, when the advantage of selfish circles is smaller than a threshold value (r near 1.3, depending on the parameters), they cannot invade and the information can be maintained. This does not seem plausible since they are more than two times smaller and contain the same number of replication origin as master circles.

In the three-level selection model (with intermolecular, intermitochondrial and intercellular selection), the maintenance of the entire mt information within cells is possible even for high values of the advantage for selfish circles (r). This maintenance is possible without a replicative advantage for the master circles, as assumed in ATLAN and COUVET's (1993) model. Although there are very few data on the way the molecules replicate, a replicative advantage for the master circle is controversial. With the exception of DE HAAS *et al.* (1993) who suggest that only master circles replicate, other authors have suggested that several molecules within mt genome can replicate (FOLKERTS and HANSON 1991; LEVY *et al.* 1991; JANSKA and MACKENZIE

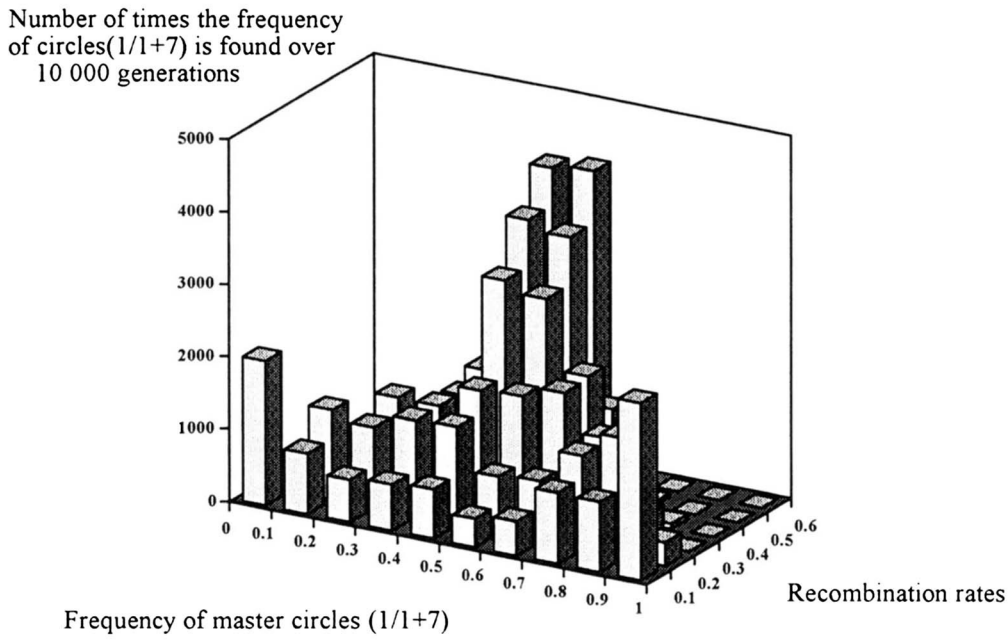

FIGURE 7.—Transitions between equilibria. The distribution of the frequencies of master circle 1 over master circles 1 and 7 are represented for 10,000 generations for different recombination rates (from 0.1 to 0.7). At high recombination rates, the two equilibria of circles cannot be observed, the circles 1 and 7 are present in the same proportions. Whereas at low recombination rates, the two equilibria of circles can be observed. This picture is quite similar to that obtained for frequency distribution at a neutral diallelic locus with different levels of drift mutation balance: when mutation is more important than drift, one observes the pattern corresponding to high recombination rates, and when drift is more important than mutation, one observes the pattern corresponding to low recombination rates. The simulation parameters are as follows: $r = 2$, $x = 5$, $N_{opt} = 3$, mt selection and truncated cellular selection.

1993). There is some evidence as well in yeast for differential replication of the mitochondrial molecules where selfish molecules replicating faster than others invade the cell by this mechanism (BLANC and DUJON 1980; DE ZAMAROCZY *et al.* 1981). The replication mechanism is still unknown. Nevertheless observations of BENDICH and SMITH (1990) on watermelon and cauliflower suggest a rolling circle mechanism generating linear molecules. Linear tails on circular molecules of all sizes has been observed, suggesting that the rolling circle mechanism is also valuable for subgenomic molecules. In our model all molecules are circular and then can replicate by a rolling circle mechanism. The mt genome is likely composed of linear and circular molecules, but linear molecules unable to become circular would not replicate with this process and thus have little effect on the dynamic of molecules.

The existence of an intermitochondrial level of selection (corresponding to intracellular selection) was pointed out using antibiotics in different organism: yeast and Paramecium (see BIRKY 1994 for a review). Antibiotic-resistant mitochondrial mutants were selected in the presence of an antibiotic. As BIRKY interpreted "when antibiotic-sensitive cells are exposed to the antibiotic, [mt] chromosomes with rare spontaneous resistant mutant genes continue to replicate until there are enough to make the cell phenotypically resistant. And when heteroplasmic cells with a mixture of resistant and sensitive genes are produced by mating, artificial cell fusion, or microinjection and are exposed to an antibiotic, they produce only homoplasmic resistant progeny". The resistant mitochondrion has replicated more than the others and has then produced a phenotypically resistant cell. That does not "prove" but is in favor of the existence of an intermitochondrial level of selection. The mutation for antibiotic resistance should not modify the replication rate of the molecule that carries it but should give an advantage to the mitochondrion containing it.

The average fitness of the cells is higher when the recombination rates are low, therefore one would expect selection at a cellular level to act for a decrease in the recombination rate. On the other hand, selection at the mitochondrial level would act for an increase of the intermolecular recombination rate. Indeed R_{inter} and R_{intra}, the two recombination rates, have an important effect on the stoichiometry of the molecules. When R_{intra} (breakdown of molecules) increases, the number of selfish molecules increases and the frequency of large selfish molecules (dimer of small selfish circles: circles 6, 14, 12) decreases. In contrast, when R_{inter} increases, the small selfish molecules decrease and the large selfish molecules increase in frequency. The average fitness of the cells decreases with an increase of R_{intra} but increases with an increase of R_{inter} and the average fitness of mitochondria decreases with an in-

crease of R_{intra} but increases with an increase of R_{inter}. This is due to the cellular limitation of resources for replication, *i.e.*, the efficiency of each replication origin. When R_{inter} increases the number of circles 6, 12, and 14 possessing two replication origins increases, therefore the total number of molecules decreases and then W_{cell} decreases.

Role of master circles and stoichiometry of mitochondrial molecules: Master circles contain all the mitochondrial information and can generate all the other molecules by recombination. Therefore, they could play an essential role in maintaining mt information. Simulations show however that this not the case: the cell fitness is not affected when the number of master circles vary. For low mitochondrial selection they can be nearly totally replaced by some combinations of molecules with a sufficient density of replication origins and a sufficient amount of coding sequences. For example, the circles 9 and 13 (Figure 2) have one replication origin and three regions containing coding sequences (as master circles), but circle 9 bears two a regions and no c region, whereas circles 13 bears two c regions and no a region. The important thing is the amount of information present in the cell whatever its organization on the molecule. From experimental data, BENDICH (1985) deduced the same idea: "it is relatively unimportant how plant mt genomes are arranged physically as long as essential sequence information is present". There is a significant correlation between the total number of circles and the fitness of the cells. Nevertheless, in our model, the proportion of master circles in cells increases with an increase of the amount of information needed for a mitochondrion to be functional. Since master circles contain the whole information in a linked state, this information remains linked from generation to generation. Therefore when the need of information to get a well-functioning mitochondrion (N_{opt}) increases, master circles increase in frequency. As SZATHMARY and MAYNARD-SMITH (1993) and MAYNARD-SMITH and SZATHMARY (1993) have demonstrated, cells with linked genes gain a large competitive advantage over the others because their offspring receives a full set of genes. Another process that would keep the information linked would be a recombination process leading to intertwined molecules, as observed in *Escherichia coli.*

When N_{opt} decreases, *i.e.*, mitochondria need less mt information to function well, the mt fitness increases for a given information content and the mtDNA will replicate more. Therefore, the less information mitochondria need, the more mtDNA molecules are present. As a consequence, there is no direct relationship between the need for mt information and the amount of mtDNA. Our model suggests that the number of circles depends on the resources of the cell and not on the need mitochondria and cells have for circles. Such a reason for the presence of high and variable numbers of mt molecules within cells is quite different from the functional explanations given by other authors. For example, BENDICH suggested in 1987 that the very high genome copy number in organelles must reflect an increasing need for organellar ribosomes that can only be satisfied by an increase in ribosomal RNA gene number that results from genome multiplication. We argue that an increase in the need for mt sequences would have more complicated consequences: selection against selfish molecules would decrease mt molecule numbers and increase the quality of the molecules (higher proportion of master circles).

Equilibria of molecules: Concerning the stoichiometry of molecules, two situations can be observed. Either it is approximately constant, or different stable stoichiometries are found. An equilibria of molecules is defined by a constant stoichiometry of the molecules over a large number of generations. When there are different equilibria, with the simple system of circles used in this study with only two master circles, four different molecular equilibria can be defined. These stable stoichiometries correspond to dynamic equilibria stable over a large number of generations. Two of them differ in the proportions of the two master circles, one being abundant and the other one in low frequency and vice versa. The other two equilibria have few master circles and a lot of circles 9 or 13 (Figure 2). The shift between equilibria is neutral (the fitness of cells is constant). The number of transition from one equilibrium to another is linked to the recombination rates. With low recombination rate, the three different equilibria defined above are well defined: the mt genome remains a long time in one state. An increase in recombination rates gives rise to an increase in transition frequency, with only one state where the two master circles are in almost equal proportions (Figure 7). As a consequence, the dynamics of the system can be understood as a drift-mutation equilibrium, mutation being the assimilating to recombination and drift corresponding to sampling fluctuations in circle numbers. Selection only defines what types of combinations are possible but has no effect on the shift from one equilibrium to another, that occurs without variations in cell fitness. The two cases (either one stable state or four equilibria with low number of transition between each other) are then similar to what happens at a neutral diallelic locus submitted to recurrent mutation and drift (ROUGHGARDEN 1979). If mutation is high relative to drift, one observes only one state, with an intermediate allelic frequency. At the opposite, if mutation is low relative to drift, one observes mainly one of the two monomorphic equilibria, and sometimes transitions occur between them (Figure 7).

Therefore equilibria can be observed when the recombination rates are under a threshold value, and if not, there is a polymorphism. This is in agreement with

the model of ATLAN and COUVET (1993), where different equilibria depending on the recombination rates were observed as well. However, in their model, some recombining sequences recombine less than others and the switch from one equilibrium to another one induces lower fitness than in the equilibrium states.

Conclusion: The intermitochondrial level of selection plays an important role in maintaining the entire mitochondrial information in cells. Therefore mitochondria should be relatively individualized during the cell cycle. Master circles are not essential for the maintenance of mt information. When the information is maintained, several equilibria of molecules can be observed. Usually the recombination rates and/or the replication rates are considered to be the only parameters involved in the change of equilibria of molecules observed experimentally. From our point of view, several processes may be responsible for the different equilibria observed. Some equilibria may be due to drift-mutation mechanisms when the effect of sampling fluctuations of circle number is more important than the effect of recombination. In addition to the role of replication and recombination rate, changes of equilibria can be due to a change in the selective pressure. This is probably observed frequently in *in vitro* culture. One example is the reversible changes in the composition of the population of mtDNA during dedifferentiation and regeneration in tobacco observed by KANAZAWA *et al.* (1994). Other changes of equilibria observed experimentally may be due to a change of the possible circles of the system: deletion or addition of sequences, which have not yet been modeled.

We thank all the students who worked on this model: N. LAFAY, S. ROZENFELD and T. CLÉROT. We thank particularly J. MAYNARD SMITH who gave a first project on this subject to B. ALBERT during an European exchange supported by an Erasmus grant. For helpful discussions and comments we thank M. HANSON, C. W. BIRKY, JR., I. SMALL, C. SMALL-COLLAS DES FRANCS, D. COUVET, F. VEDEL and C. LELANDAIS.

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Communicating editor: M. R. HANSON