THE last few years have witnessed the accumulation of a considerable body of experimental data dealing with the isolation of mitochondrial mutants in yeast conferring resistance to various inhibitors and with the results of two, three or four factor crosses using these mutants as genetic markers. All these results can be classified as belonging to two types of experimental approaches: one based essentially on an analysis of the progeny of an individual cell heterozygous for mitochondrial genetic markers and the second based essentially on an analysis of the progeny of a population of cells heterozygous for mitochondrial genetic markers. The first approach is exemplified by the work of Linnane et al. 1968; Thomas and Wilkie 1968; Coen et al. 1970; Saunders et al. 1971; Lukins et al. 1973; Wilkie and Thomas 1973; the second by that of Coen et al. 1970; Bolotin et al. 1971; Kleese, Grotbeck and Snyder 1972 a and b; Rank and Bech-Hansen 1972; Avner et al. 1973; Avner and Griffiths 1973; Howell et al. 1973; Rank 1973; Wakabayashi and Kamei 1973; Wolf, Dujon and Slonimski 1973; Netter et al. 1974; Coen et al. (in preparation).

At the very start of this period of mitochondrial genetics it became apparent that individual zygotic clones issued from a cross of two mitochondrally pure lines may behave quite differently from one another with respect to the types and proportions of the mitochondrial genotypes they give rise to (Coen et al. 1970). Pedigree analyses performed since have shown that not only are the proportions of different cell types variable from clone to clone but also their order of appearance (Lukins et al. 1973; Wilkie 1973). Apart from the demonstration of inter- and intraclonal heterogeneity as one of the basic features of mitochondrial crosses, reminiscent of course of the heterogeneity in burst size and composition in bacteriophages crosses, this “individualistic” approach has not as yet yielded any substantial insights into the mechanism of mitochondrial genetics.

The “population” approach is based on what Coen et al. (1970) have called a standard cross and has been adopted with no or only minor modifications by several authors (Bolotin et al. 1971; Kleese, Grotbeck and Snyder 1972 a and b; Rank and Bech-Hansen 1972; Suda and Uchida 1972; Avner and Griffiths 1973; Avner et al. 1973; Howell et al. 1973; Rank 1973; Trembath et al. 1973; Wakabayashi and Kamei 1973; Wolf, Dujon and Slonimski 1973; Netter et al. 1974 and Coen, in prep.). The standard cross is performed in such a way that (1) a sample of diploid cells randomly collected at the end of the cross should

be representative both of a large number of zygotes formed by the fusion of haploid cells at the onset of the cross and of a large number of diploid cells issued from such zygotes by vegetative multiplication; (2) the rate of cell multiplication should be identical for all the cells and should not be influenced by the nature of their mitochondrial genomes; that is no selective advantage at the cellular level should occur; (3) the various cell types should be genetically pure at the end of the cross with respect to their mitochondrial genomes. Under ideal conditions the frequencies of the various cell types found in the population should be a good estimate of the frequencies of the various mitochondrial DNA molecules issuing from the cross.

The systematic application of this methodology has led to the description of a certain number of features characteristic of mitochondrial crosses (Coen et al. 1970; Bolotin et al. 1971; Kleese, Grotbeck and Snyder 1972b; Avner et al. 1973; Wolf, Dujon and Slonimski 1973; Netter et al. 1974; Coen et al. in prep.). However, no unifying view integrating these different features has as yet emerged. Such a unification is the purpose of this article. A model which is aimed at incorporating all the facts presently known about mitochondrial crosses in yeast has been developed. This model will be presented in the second part of this article, the first part being devoted to a summary of the experimental data.

GENETIC FEATURES OF MULTIFACTORIAL CROSSES AND THEIR IMPLICATIONS

1) Mutations mapped on the mit-DNA

Figure 1 gives a list of some mutations for which the localization on the mitochondrial DNA has been demonstrated. Mutations have been isolated with the help of drugs to which they confer resistance. Evidence in favour of their localization on mit-DNA is based on the fact that they are included in the \( \rho^+ \) factor (cf. Deutsch et al. 1974), on the analysis of changes in mitochondrial DNA sequences in relation to the physical deletion and repetition of various segments of the molecule (cf. Faye et al. 1973) and on allelism and recombination studies between mutations. Six genetic loci are known at present. Three loci \( R_\text{I}, R_\text{II} \) and \( R_\text{III} \) are linked and are located in a segment specifying the mitochondrial ribosome (Grivell et al. 1973; Netter et al. 1974). The other three loci are genetically unlinked to each other and are also unlinked to the ribosomal segment of mit-DNA. Loci \( O_\text{I} \) and \( O_\text{II} \) confer resistance to oligomycin and specify mitochondrially synthesized components of the membrane ATPase complex (Avner et al. 1973; Somlo et al. 1974). The gene product of the paromomycin-conferring-resistance locus is less well known but circumstantial evidence suggests that it also acts at the mitoribosomal level (Wolf, Dujon and Slonimski 1973). Mutants belonging to the same locus are probably heteroalleles although some of them could concern the same mutational site. In some cases (especially for the loci \( R_\text{I}, R_\text{II} \) and \( R_\text{III} \)) it has been shown that mutants mapping at the same locus confer different resistant phenotypes and must therefore represent non-identical modifications of the mit-DNA base sequence (Slonimski et al. in prep.).
FIGURE 1.—Mutations located on the mitDNA. The data are compiled from Coen et al. (1970), Bolotin et al. (1971), Avner et al. (1973), Wolf, Dujon and Slonimski (1973), Netter et al. (1974) and Coen et al. in prep. *CR, ER, SR, OR, or PR* with subscripts represent resistant mutations originally isolated on media containing respectively chloramphenicol, erythromycin, spiramycin, oligomycin or paromomycin. Each subscript represents an independently isolated mutation. $\omega^+$; $\omega^-$; $\omega^N$ are allelic forms of the mitochondrial locus $\omega$. (*) $C^R$ mutations associated with an $\omega^N$ mutation at the $\omega$ locus (see text).

2) Segregation of mitochondrial genomes during mitotic cell divisions

Mitochondrial genes are characterized by the segregation occurring in clones during the vegetative phase of cellular multiplication. Clones heterozygous for mitochondrial genes are referred to as “heteroplasmons” (Von Wettstein 1928), “heterocytons” (Catcheside 1958), or “cytohets” (Sager 1972). The simplest interpretation is that mitochondrial DNA molecules are sorted out between the mother cell and the bud at each cell division. Such segregation leads eventually to the formation of pure cells, that is cells breeding true to type and homozygous for mitochondrial genes (“homoplasmons,” etc. . . ). Although
many questions concerning both the manner and the rules which govern the segregation of mit-DNA molecules are unanswered at the present time, it is well established in the case of yeast, that heteroplasmons do not last for a considerable number of cell divisions, and that segregation leads rapidly to the formation of pure cell lines. This was first shown by Coen et al. (1970) who found that genetically pure recombinant cells are already formed during the early divisions of a zygote. More recently we have performed microdissection experiments in order to obtain quantitative estimates on the segregation process. The first buds issued from zygotes of a three factor cross $o^+ C^8 R^8 O^8 \times o^+ C^8 E^8 O^R$ have been separated by micromanipulation and allowed to form clones on non-selective medium. Samples of ca. $5 \times 10^5$ cells of each clone have then been tested for the presence or absence of resistant cells by dropping out on media containing either chloramphenicol or erythromycin or oligomycin. Among 74 first bud clones scored, 29 were found not to contain a single $O^8$ cell, these first buds were therefore pure $O^8$. Similarly two first buds were pure $C^8$ and two were pure $E^8$. From considerations which will be presented in a later part of this article it was estimated that the input fraction of mit-DNA molecules carrying the $O^8$ allele was close to $2/3$ in this particular cross and the input fraction of mit-DNA molecules carrying the $C^8$ and $E^8$ alleles was $1/3$. Assuming an intentionally oversimplified model of randomly distributed mit-DNA molecules it can be deduced from these data by a straightforward calculation of the type:

$$P = (w)^n$$

where $P$ is the probability of forming a pure bud (either $O^8$ or $C^8$ or $E^8$)

- $w$ is the input fraction of the allele considered
- $n$ is the number of molecules entering the bud

that very few mit-DNA molecules enter the bud from the zygote (around 3 on the average).

$$P_{O^8} = \left(\frac{2}{3}\right)^3 = 0.30 \approx \frac{29}{74}$$

$$P_{C^8,E^8} = \left(\frac{1}{3}\right)^3 = 0.04 \approx \frac{2}{74}$$

This estimate should be treated with caution because nothing is known about the way in which mit-DNA molecules are organized within organelles and the distribution process itself may not be random. Furthermore, if only a few molecules enter the bud, there must exist a mechanism which rapidly increases this small number to the statistical average of some 50 to 100 molecules per cell. Our ignorance on this subject is complete. Finally the effects of genetic exchanges, such as gene conversion processes, by tending to increase homozygosity within the cells, will contribute to the increase in the frequency of pure cells. The experimental data, however, indicate clearly that segregation is rapid and that mitochondrially pure homozygotic cell lines are produced after a relatively small number of cell divisions.
3) **Recombination and the \( w \) locus**

The reassortment of mitochondrial genes has been shown to occur in yeast by Thomas and Wilkie (1968). Postmeiotic stability and backcrosses have demonstrated that the reassortment resulted from genetic recombination (Coen et al. 1970; Bolotin et al. 1971). More recently Michaelis, Petrochilo and Slonimski (1973) have analyzed the molecular basis of genetic recombination and have shown that it involves the formation of physically recombined mit-DNA molecules.

Simultaneously with the demonstration of genetic recombination between mitochondrial genes two characteristic features of mitochondrial crosses have become conspicuous (Coen et al. 1970; Bolotin et al. 1971): a bias in the transmission of alleles and a bias in recombinant frequencies. The first describes the fact that, in some crosses, two alleles of the same locus (one from each parent) do not appear with equal frequencies among the progeny of a mitochondrial cross. The second relates to the observation that, in some crosses, one recombinant type between two markers appears with a much greater frequency than its reciprocal type (e.g., the major recombinant is ca 100 times more frequent than the minor recombinant in crosses involving \( C \) and \( E \)). All crosses which show a strong bias in the frequency of reciprocal recombinants display thereby a bias in the transmission. The converse is not however true. There exist crosses which show a bias in transmission without showing any bias in the frequency of recombinants. A certain amount of confusion concerning these two phenomena and their causal relations has appeared in the literature recently (Howell et al. 1973, Rank and Bøgh-Hansen 1972). To avoid any misunderstanding we have adopted the following nomenclature. "Whenever amongst the diploid progeny of a cross there is found a bias in the distribution of two alleles of a mitochondrial gene, or a bias in the frequency of the two reciprocal recombinants between two non allelic mitochondrial genes is observed, the term polarity should only be used when the genetic determinant responsible is known to be localized on the mitochondrial genome. When a nuclear gene(s) is implicated as the determinant, then the term asymmetry should be used, whilst in cases where the nature of the genetic determinant responsible for the bias remains unknown a third term such as bias should be applied. Until now the only known genetic determinant responsible for the polarity phenomena is the specific mitochondrial locus \( w \) (Avner et al. 1973)." The \( w \) locus is closely linked to the ribosomal segment (cf. Figure 1). Among all the different wild-type yeast strains we have studied until now only two allelic forms of \( w \) (\( w^+ \) and \( w^- \)) have been found (Coen et al. in prep.) For this reason Bolotin et al. (1971) have designated \( w \) as the mitochondrial sex locus. Each yeast cell carries either the \( w^+ \) allele or the \( w^- \) allele. The nature of the allele carried at the \( w \) locus by a given strain is detected by crossing it to tester strains. When a significant polarity of recombination is observed in the ribosomal segment of mit-DNA, the cross is designated as a heterosexual one between two strains, one \( w^+ \), the other \( w^- \). On the other hand, crosses between two strains carrying the same \( w \) allele (\( w^+ \times w^+ \) or \( w^- \times w^- \)) never show any significant polarity of recombination.
for the ribosomal segment (see Bolotin et al. 1971 and Coen et al. in prep.) and are referred to as homosexual crosses. A third allelic form (\(\omega^N\)) of the \(\omega\) locus has been isolated amongst some spontaneous mutants at the \(R_I\) locus. \(\omega^+\rightarrow \omega^N\) mutations occur with a very low frequency (\(\approx 10^{-7}\)). The \(\omega^N\) allelic form is characterized by the fact that no significant polarity of recombination is observed both when an \(\omega^N\) strain is crossed not only with an \(\omega^+\) strain but also with an \(\omega^-\) strain (Dujon et al. in prep.)

4) Multifactorial crosses

The main features of multifactorial mitochondrial crosses have been described in detail in publications from this laboratory (Coen et al. 1970; Bolotin et al. 1971; Avner et al. 1973; Wolf, Dujon and Slonimski 1973; Netter et al. 1974; Coen et al. in prep.) and by Kleese, Grotbeck and Snyder 1972b, Howell et al. 1973; Rank 1973. The model proposed in the present work is based entirely on the experimental data obtained at Gif, but is equally applicable to that obtained by Kleese, Grotbeck and Snyder 1972b; Howell et al. 1973 and Rank 1973. It seemed to us appropriate to give two examples of mitochondrial crosses before entering into discussions concerning the model. With the help of these two examples we shall point out the salient characteristics of mitochondrial genetics. The examples are given in Table 1 and concern a homosexual cross and a heterosexual cross involving the three unlinked markers \(C\), \(O\) and \(P\).

4.1) Polarity: The heterosexual cross displays a significant polarity of recombination so long as the \(C\) marker is involved (\(C^+O^-/C^-O^+ = 46.6/0.6 = 77; C^+P^-/C^-P^+ = 41.5/0.6 = 68.5\)). On the other hand no significant polarity is observed between the \(O\) and \(P\) markers (\(O^+P^-/O^-P^+ = 7.9/13.0 = 0.6\)). This observation has led us to make a distinction between markers located in polar regions and markers located in nonpolar regions. The loci \(R_I\), \(R_{II}\) and \(R_{III}\) are located in the polar region while the loci \(O_I\), \(O_{II}\) and \(P_I\) are located in nonpolar regions. The polar region is linked to \(\omega\). The homosexual cross displays no significant polarity of recombination for any of the pair of markers considered (\(C^O^+/C^O^- = 13.3/10.9 = 1.2; C^P^+/C^P^- = 7.8/8.3 = 0.9; O^P^+/O^P^- = 9.8/12.7 = 0.8\)). This absence of polarity of recombination is true not only for the markers listed in Table 1 but for all the markers presently known.

4.2) Output and input of alleles: We have mentioned previously that two alleles of the same locus (one from each parent) are not necessarily transmitted with equal frequency to the progeny of a mitochondrial cross. We shall refer to the frequency of a given allele among the total population as the output fraction. Similarly, we shall refer to the frequency of that allele among the total population of mit-DNA molecules at time zero of the cross, that is immediately after the fusion of two haploid cells leading to the formation of the zygote as the input fraction. The output fraction is a parameter that can be determined experimentally by measuring the frequency of a given cell type among the total cell population. The input fraction is a parameter which cannot be determined directly by experimentation. It can be deduced however, from the output fraction on the basis of the following evidence.
**TABLE 1**

*Examples of two three point crosses with the markers C, O and P*

<table>
<thead>
<tr>
<th>Cell type</th>
<th>HETEROSEXUAL CROSS</th>
<th></th>
<th>HOMOSEXUAL CROSS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KL14-4A</td>
<td>IL102-5C</td>
<td>KL14-4A</td>
<td>IL215-1B</td>
</tr>
<tr>
<td></td>
<td>$a^+C_{+}O_{+}P_{+}$</td>
<td>$a^+C_{+}O_{+}P_{+}$</td>
<td>$a^+C_{+}O_{+}P_{+}$</td>
<td>$a^+C_{+}O_{+}P_{+}$</td>
</tr>
<tr>
<td>Observed colonies (percent)</td>
<td>123</td>
<td>(37.3)</td>
<td>175</td>
<td>(32.1)</td>
</tr>
<tr>
<td>Predicted colonies (percent)</td>
<td>118</td>
<td>(35.7)</td>
<td>165</td>
<td>(30.3)</td>
</tr>
<tr>
<td>Observed colonies (percent)</td>
<td>26</td>
<td>(7.9)</td>
<td>15</td>
<td>(2.8)</td>
</tr>
<tr>
<td>Predicted colonies (percent)</td>
<td>39</td>
<td>(11.8)</td>
<td>30</td>
<td>(5.5)</td>
</tr>
<tr>
<td>Observed colonies (percent)</td>
<td>43</td>
<td>(13.0)</td>
<td>45</td>
<td>(8.3)</td>
</tr>
<tr>
<td>Predicted colonies (percent)</td>
<td>39</td>
<td>(11.8)</td>
<td>32</td>
<td>(5.5)</td>
</tr>
<tr>
<td>Observed colonies (percent)</td>
<td>111</td>
<td>(33.6)</td>
<td>27</td>
<td>(5.0)</td>
</tr>
<tr>
<td>Predicted colonies (percent)</td>
<td>105</td>
<td>(31.8)</td>
<td>32</td>
<td>(5.9)</td>
</tr>
<tr>
<td>Observed colonies (percent)</td>
<td>2</td>
<td>(0.6)</td>
<td>21</td>
<td>(3.9)</td>
</tr>
<tr>
<td>Predicted colonies (percent)</td>
<td>1</td>
<td>(0.2)</td>
<td>30</td>
<td>(5.5)</td>
</tr>
<tr>
<td>Observed colonies (percent)</td>
<td>0</td>
<td>(0.0)</td>
<td>38</td>
<td>(7.0)</td>
</tr>
<tr>
<td>Predicted colonies (percent)</td>
<td>1</td>
<td>(0.2)</td>
<td>33</td>
<td>(5.9)</td>
</tr>
<tr>
<td>Observed colonies (percent)</td>
<td>0</td>
<td>(0.0)</td>
<td>24</td>
<td>(4.4)</td>
</tr>
<tr>
<td>Predicted colonies (percent)</td>
<td>0</td>
<td>(0.2)</td>
<td>32</td>
<td>(5.9)</td>
</tr>
<tr>
<td>Observed colonies (percent)</td>
<td>25</td>
<td>(7.6)</td>
<td>200</td>
<td>(36.7)</td>
</tr>
<tr>
<td>Predicted colonies (percent)</td>
<td>26</td>
<td>(7.9)</td>
<td>545</td>
<td>(35.6)</td>
</tr>
<tr>
<td>Total</td>
<td>330</td>
<td></td>
<td>545</td>
<td></td>
</tr>
</tbody>
</table>

**Input fraction**

(Average $O^a$, $P^a$) 0.48 0.48 0.47 0.47

<table>
<thead>
<tr>
<th>Total recombinants (percent)</th>
<th>47.3</th>
<th>44.2</th>
<th>24.0</th>
<th>22.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>42.1</td>
<td>44.2</td>
<td>16.0</td>
<td>22.7</td>
</tr>
<tr>
<td>OP</td>
<td>20.9</td>
<td>24.2</td>
<td>22.4</td>
<td>22.7</td>
</tr>
<tr>
<td>Polarity and limits</td>
<td>32</td>
<td>72</td>
<td>12 (0.9–1.6)</td>
<td>1</td>
</tr>
<tr>
<td>CP</td>
<td>28</td>
<td>72</td>
<td>0.9 (0.7–1.3)</td>
<td>1</td>
</tr>
<tr>
<td>OP</td>
<td>0.6</td>
<td>1</td>
<td>0.8 (0.6–1.0)</td>
<td>1</td>
</tr>
</tbody>
</table>

Two three point crosses (C, O and P) are presented, a cross between two $\omega^+$ strains (homo-sexual) and a cross between an $\omega^+$ and an $\omega^-$ strain (heterosexual). For each cross two sets of numbers are given. One set corresponds to the actual number of colonies counted. (Data are taken from Tables 6b and 11b in WOLF, DJON and SLONIMSKI 1973). The other set corresponds to the number of colonies predicted by the model. The calculation is developed in the text.

4.3) **Coordinate output**: The output fraction of a given allele is a reproducible characteristic of a cross performed under a given set of conditions. Different crosses or different conditions lead to different output fractions of the same allele and these values vary from almost zero to almost one. The main feature of this variation is a complete correlation between the output fractions of alleles coming from the same parent and belonging to two different loci. This phenomenon which is referred to as coordinate output or covariance of transmission is exemplified by the data concerning loci C and O taken from a series of homosexual crosses and presented in Figure 3a. Table 1 also gives an example where the output fraction...
of allele coming from the $a$ parent is 0.48 for the $C$ mitochondrial gene, 0.46 for the $O$ gene and 0.49 for the $P$ gene. These three values are not statistically different and the average output fraction for mitochondrial alleles coming from the $a$ parent is 0.47. A strict correlation between output fractions between any pairwise combination of alleles at the loci $R_I$, $R_{II}$, $R_{III}$, $O_I$, $O_{II}$ and $P_I$ has been demonstrated in homosexual crosses. Such a correlation strongly suggests that the variations in output are due to the variations in the input of mit-DNA molecules and that all the genetic loci showing this correlation are carried on the same mit-DNA molecule. In heterosexual crosses coordinate output is observed between markers located in nonpolar regions such as $O$ and $P$. As in the homosexual crosses, we shall consider therefore, that in heterosexual crosses the output fraction of alleles located in nonpolar regions is an adequate estimate of the input fraction of mit-DNA molecules. The situation is quite different for markers located in a polar region such as $C$, $S$ and $E$. In this case the phenomenon of polarity of recombination due to $a$ modifies the output fraction in such a way that the output fraction is no longer proportional to the input fraction. In heterosexual crosses the output fractions of markers located in the polar region are simultaneously a function of both their input fractions and the phenomenon of polarity. Figure 3b shows the relationship between the output fraction of the $C$ marker and that of the markers in the nonpolar regions.

4.4) **Upper limit of recombinant frequencies in homosexual crosses:** The frequency of recombinants between different markers varies according to the pair of markers considered. There is however an upper limit close to 20–25%. The sum total of recombinants between markers $CO$, $CP$, $EO$, $EP$, $OP$ etc. is close to that limit, while it is lower between markers of the ribosomal segment. This suggests that the value of 20–25% recombinants amongst the total population is the limit for the recombinant frequency between two genetically unlinked markers carried on the same mit-DNA molecule. Three and four point crosses have shown that markers located at the loci $O_I$, $O_{II}$ and $P_I$ are unlinked to the markers of the ribosomal segment and unlinked to each other. Table 1 shows that the recombinant frequency is 24.0% for $CO$, 16.0% for $CP$ and 22.4% for $OP$. The fact that the upper limit for unlinked markers is significantly lower than 50% has led us to the idea that there is a panmictic pool of molecules, in which both homologous and heterologous pairings occur, the former pairing events not however being genetically detectable.

4.5) **Positive coincidence in homosexual crosses:** The idea of a panmictic pool of mit-DNA molecules is further confirmed by the fact that a strong positive coincidence (2- to 3-fold) exists for all the mitochondrial genome in homosexual crosses. Table 1 shows an example of such positive coincidence. Three orders are possible $C-O-P$, $C-P-O$ and $P-C-O$. The ratio of the frequency of double recombinants over the product of frequencies of single recombinants is greater than two for the three postulated orders $C-O-P$, $C-P-O$ and $P-C-O$. Such a positive coincidence can be fully explained if there is a panmictic pool of molecules.

4.6) **Excess of recombinants in heterosexual crosses:** In heterosexual crosses the sum total of the two reciprocal types of recombinants between two markers
(the major recombinant plus minor recombinant) is greater than the upper limit observed in homosexual crosses. Such a phenomenon can be seen in Table 1 where the frequency of the recombinants between $C$ and $O$ is 47.3% and that between $C$ and $P$ is 42.1% in the heterosexual cross while it is respectively 24.0% and 16.0% in the homosexual cross. The excess of recombinants is related to the polarity of recombination. The greater the polarity of recombination the greater the frequency of recombinants. This suggests that polarity is correlated to an obligatory recombination event able to produce recombinants in excess of the usual limit. This obligatory event would occur every time a pairing between an $\omega^+$ and an $\omega^-$ genome took place and would produce one type of recombinant only, the major one, and not the reciprocal one.

4.7) Polarity in the ribosomal segment amongst recombinants between other markers: To explain our original results on the polarity of recombination observed between the two markers, $C$ and $E$, a hypothesis of a sequential transfer of DNA from $\omega^+$ mitochondria into $\omega^-$ mitochondria was advanced by Boilotin et al. (1971). This transfer was supposed to be initiated at the $\omega^+$ locus itself and the excess of the $C+E^-$ recombinant was supposed to result from an interruption of the transfer and the elimination of the $E^+$ allele from the mating occurring in mitochondrial meiosis. As soon as other mutants were isolated such as oligomycin-resistant (Avner et al. 1973) and paromomycin resistant ones (Wolf, Dujon and Slonimski 1973), four point crosses were performed. It became feasible in such crosses to compare the polarity of recombination between two markers among the total population on the one hand and simultaneously among the subpopulation of recombinants for the two other markers on the other hand. Wolf, Dujon and Slonimski (1973) have shown that the polarity of recombination between $C$ and $E$ is similar when measured among the total population and when measured among the subpopulation which have recombined between $O$ and $P$. In a sequential transfer hypothesis $O$ and $P$ would have been distal markers and $C$ and $E$ proximal ones with respect to the initiation point. Therefore no polarity would have been expected between $C$ and $E$ among recombinants for distal markers. The fact that the polarity does persist excludes the sequential transfer hypothesis.

4.8) UV effects: Ultraviolet irradiation of one parent prior to mating with an unirradiated parent has been used as a tool to study mitochondrial crosses and the output of markers at different loci, as a function of UV dose, has been systematically measured. Boilotin et al. (1971) have reported first, in the case of heterosexual crosses, that the output of the marker $C$ is much less influenced by increasing doses of UV than the output of the marker $E$. Further experiments have confirmed this observation and have shown that: (1) in homosexual crosses the output of markers at the different loci is equally affected by UV; (2) in heterosexual crosses the output of markers located in nonpolar regions is affected by UV in a manner exactly similar to that of the same markers in homosexual crosses. On the contrary, the markers located in the polar region in heterosexual crosses are much less influenced by UV than the markers located in nonpolar regions. The closer the location of a marker to $\omega$ the less the effect of UV on its
in homosexual crosses the frequency of recombinants decreases when a bias in the output is introduced by UV irradiation of either one of both parents. In heterosexual crosses the frequency of recombinants increases when a bias in the outputs is introduced by UV irradiation of the $\omega^+$ parent, and the output of the $\omega^-$ increases.

All these observations are compatible with the idea that the effects of UV concern the input fraction almost exclusively. Irradiation inactivates or withdraws from the mating pool a certain number of mit-DNA molecules of the irradiated cell and therefore diminishes the input fraction of genomes introduced by that cell into the zygotes. The effects on the frequency of recombinants are in agreement with those predicted by a panmictic pool of molecules and the differential effect on the survival of markers can be fully interpreted by considering the relationship between the output of the $C$ and $E$ markers as function of the input fraction (see Figure 3b).

4.9) Distribution of the third marker among recombinants between two others: In three point crosses it is possible to look at the distribution of one given marker among recombinants between two others and to compare such a distribution to the distribution of the same marker in the overall population. In homosexual crosses such a comparison clearly establishes the linkage between markers of the ribosomal segment while it shows the absence of linkage between other markers. In heterosexual crosses the distribution of a third marker among recombinants for two markers located in nonpolar regions is the same as among the total population. Among the two types of reciprocal recombinants between markers located in the polar region an excess of the allele of the third marker originating from the $\omega^-$ parent is observed.

A MODEL FOR PAIRING AND RECOMBINATION BETWEEN MIT-DNA MOLECULES

We shall now develop a theory aimed at explaining the characteristic features of the mitochondrial crosses described in the preceding chapter. Only the main ideas of the model will be discussed here with some examples of the calculations and the predictions. The complete theory including the mathematical and statistical calculations as well as the details of the computer analysis of the experimental results will be presented in a separate publication (DUJON, in preparation).

The model is composed of two parts: one dealing with the process of pairing between mitochondrial DNA molecules and the other dealing with the mechanism of recombination between paired mit-DNA molecules. The first part has been inspired by the VISCONTI and DELBRÜCK model for bacteriophage crosses (1953). The second part aims more specifically to explain the salient features of mitochondrial crosses in yeast.

In this chapter the premises of the model will be presented without discussion. The predictions of the model will be compared with the experimental data in the next section.
1) Pairing of mitochondrial DNA molecules

1.1) Panmictic pool of molecules:

There are many molecules of mit-DNA per yeast cell. Let us assume that they are all genetically complete, identical and competent and that they form a panmictic pool of molecules. Homozygous pairings occur as well as heterozygous pairings. Any pair of molecules can undertake the same kind of molecular exchanges whether genetically homozygous or genetically heterozygous. The genetically detectable events are only those which occur between heterozygous pairs.

1.2) Input fraction: The panmictic pool of molecules is characterized by the proportion of the molecules of each parent at time zero of the cross (when the two cells fuse). The proportion of molecules of one parent relative to the total number of molecules is the input fraction. This input fraction is a characteristic parameter of each cross under a given set of experimental conditions. It varies from cross to cross from 0 to 1. In the absence of any selective advantage, either at the intermolecular level within a cell or at the intercellular level within a clone, the output fraction of molecules will be proportional to the input fraction. The output fraction under such ideal conditions measures the input fraction and is also variable from one mitochondrial cross to another between 0 and 1.

1.3) Several random-in-time mating rounds: The panmictic pool of molecules can undertake several random-in-time mating rounds. There are several pairing events in the line of ancestry of an average mit-DNA molecule therefore before it is sorted out into a pure cell. The genetic composition of the pool evolves as a function of the number of mating rounds.

2) Genetic and molecular recombination events and the evolution of the pool

2.1) Types of the elementary act of recombination: Let us consider first the elementary act of recombination between two paired molecules. A clear-cut distinction has to be made between the products of a single elementary act of recombination and the products of several rounds of mating and several acts of recombination among the total population of molecules.

Secondly let us consider a situation where two reciprocal recombinants appear with equal frequencies in the population. Such a result can be explained either by a reciprocal elementary act of recombination or by a non-reciprocal elementary act. In the latter case each elementary act produces only one type of recombinants. However, one elementary act produces one type of recombinants and another elementary act produces the reciprocal type. Statistically, the two reciprocal recombinant types are produced with equal frequencies. The main difference between these two mechanisms is that the first one produces two recombinant molecules while the second one produces one parental molecule along with one recombinant molecule per elementary act. The second mechanism therefore requires twice as many mating rounds to produce the same number of recombinant molecules as the first mechanism.

Lastly let us consider a situation in which two reciprocal recombinants appear at the population level with drastically unequal frequencies. In this case the ele-
**Figure 2.**—Schematic representation of the statistically dissymmetrical gene conversion occurring in the polar region adjacent to the ω locus. The C, S and E markers represent respectively the \( R_{ii} \), \( R_{III} \) and \( R_{HII} \) loci which are genetically linked and specify the mitochondrial ribosome. They are located in a polar region under the control of ω. Other markers such as \( O \) and \( P \) are located in nonpolar regions and are genetically unlinked to the polar region. They are symbolized nonspecifically by X and Y.——Each double stranded DNA molecule of the pair is represented by a bar. Solid and open bars indicate source of genetic information, not material. Solid bar: \( ω^+ \) parent; open bar: \( ω^- \) parent. Pairing between \( ω^+ \) and \( ω^- \) alleles specifically initiates a gene conversion process by an obligatory excision of the \( ω^- \) allele followed by a sequential degradation of the \( ω^- \) sequence and a resynthesis using the \( ω^+ \) sequence as template. The final outcome of this process is a double stranded molecule presenting a segment of \( ω^+ \) sequence (solid bar) and a segment of the \( ω^- \) sequence (open bar). At every point the gene conversion process can be arrested with a certain probability \((1-\delta)\). Therefore, the probability of gene conversion \( ω^- → ω^+ \) is greater than that of \( C^- → C^+ \), greater than that of \( S^- → S^+ \) and greater than that of \( E^- → E^+ \).

This scheme does not take into account the double stranded structure of DNA molecules. In particular, it does not consider which strand is degraded and in which direction, whether strands exchange or not during the process, what the enzymes involved are etc. . . . It simply points out that: (1) there is a finite probability of converting one sequence into a copy of the other and that at the end of the process one allele has disappeared while the other is represented twice; (2) the products of the process are always one parental molecule (the \( ω^+ \) one) and one recombinant molecule.

**Table:**

<table>
<thead>
<tr>
<th>HETEROSEXUAL PAIRING EVENT</th>
<th>SEQUENTIAL UNIDIRECTIONAL DISSYMMETRICAL GENE CONVERSION PROCESS</th>
<th>MOLECULAR PRODUCTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>non polar region</td>
<td>probability of stopping the gene conversion process</td>
<td>molecular products</td>
</tr>
<tr>
<td>non polar region</td>
<td></td>
<td></td>
</tr>
<tr>
<td>polar region</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Diagram:**

- The diagram shows the statistical dissymmetrical gene conversion process in the polar region adjacent to the \( ω \) locus.
- The process involves the \( ω^+ \) and \( ω^- \) alleles, with the \( ω^- \) allele being excised, degraded, and resynthesized using the \( ω^+ \) sequence as a template.
- The final outcome is a double-stranded molecule with \( ω^+ \) and \( ω^- \) sequences.
- The process can be arrested with a certain probability \((1-\delta)\).
- The molecular products consist of one parental molecule (\( ω^+ \)) and one recombinant molecule.

**Commentary:**

- The elementary act of recombination has to be a non-reciprocal one and statistically it must always occur, in the population of molecules, in the same direction.

**2.2) Elementary act of recombination in the polar region:** The model is based on the idea that in the polar region of heterosexual crosses the acts of recombination are both non-reciprocal at the elementary level and dissymmetrical at the
population level. In other words, every time an $\omega^+$ molecule pairs with an $\omega^-$ one an obligatory event of recombination takes place, initiated always at the $\omega$ locus and always taking the same direction. Such a process produces as many parental as recombined molecules in each mating round, but only one type of recombinants, the major type, is produced. Figure 2 gives a schematic and intentionally over-simplified representation of this process.

2.3) **Consequences of the type of elementary act of recombination in relation to the number of mating rounds:** The model postulates that the number of mating rounds in the line of ancestry of an average mit-DNA molecule before its sorting out into a pure cell is the same for both the polar and nonpolar regions in heterosexual crosses. It postulates also that the average number of mating rounds is the same in homosexual and heterosexual crosses. Given these premises, a logical conclusion for heterosexual crosses is that, in nonpolar regions, the act of recombination has to be non-reciprocal at the elementary level but statistically symmetrical at the population level. Otherwise, the number of mating rounds for polar regions would have to be much greater than for nonpolar regions. Given the fact that the frequency of recombinants for nonpolar regions in heterosexual crosses is similar, if not identical, to the frequency of recombinants for the same region in homosexual crosses it can be concluded that the acts of recombination in homosexual crosses are non-reciprocal at the elementary level but symmetrical at the population level. Otherwise, the number of mating rounds in homosexual crosses would have to be different from the number of mating rounds in heterosexual crosses.

In summary: all regions of mit-DNA molecules undergo a non-reciprocal recombination at the elementary level. In homosexual crosses as well as in nonpolar regions in heterosexual crosses, these elementary acts occur at the population level in opposite directions with equal frequencies, while in the polar region of heterosexual crosses they occur always in the same direction beginning at the same point, the $\omega$ locus.

**Predictions of the Model Compared with Experimental Data**

1) *Theoretical calculation of the frequencies of molecules in the pool:* From the model described in the preceding section it is possible to calculate the frequency of every type of mit-DNA molecule, parental and recombinant, after any number of mating rounds when the input fraction and the probabilities of genetic exchanges are known.

Let us describe one of the simplest examples. Consider a heterosexual cross $\omega^+C^+O^+ \times \omega^-C^-O^-$. The $C$ marker is located in a polar region and the $O$ marker is located in a nonpolar region; $C$ and $O$ are genetically unlinked. In such a cross five molecular types are present in the panmictic pool: the two parental types $\omega^+C^+O^+$ and $\omega^-C^-O^-$ plus the three possible recombinant types $\omega^+C^-O^+$, $\omega^-C^+O^-$ and $\omega^-C^-O^-$. There are only 3 recombinant types instead of 6 because the model postulates that the conversion at the $\omega$ locus is an obligatory process, that is any pairing between an $\omega^+$ and an $\omega^-$ molecule produces always two $\omega^+$ molecules.

Let $y_1, y_2, y_3, y_4$ and $y_5$ be respectively the frequencies of $\omega^+C^+O^+$, $\omega^+C^-O^-$, $\omega^-C^+O^+$, $\omega^-C^-O^-$ and $\omega^-C^-O^-$ molecules within the pool at any time. At zero time
(initial conditions) $y_1$ is equal to the input fraction of the $\omega^+C^+O^+$ molecules, $y_5$ is equal to that of $\omega^-C^-O^-$ molecules and all the others values are equal to zero. Within the pool any pairing can occur and the probability of a pairing between two molecules is the product of the frequencies of the two molecules. Consider now, as an example, a pairing event of the type $\omega^+C^+O^- \times \omega^-C^+O^-$. The probability of such a pairing will be equal to $2y_2y_5$. In such a pairing the specific gene conversion is initiated at the $\omega$ locus and every product will be $\omega^+$. Then the gene conversion can proceed in one direction (say from left to right). The probability of arresting the process before reaching $C$ is $(1-s_2)$. In that case the products will be $\omega^+C^+O^-$ and $\omega^+C^-O^-$. When the gene conversion reaches $C$ (probability $s_2$) the final products will be $\omega^+C^+O^-$ and $\omega^+C^-O^-$. The elementary change in frequencies of each molecular type introduced in the pool by such a pairing can be calculated as follows:

\[
\frac{dy_2}{dm} = 2y_2y_5\left(\frac{1}{2} - \frac{1}{2} s_2\right) = y_2y_5s_2
\]

\[
\frac{dy_4}{dm} = 2y_4y_5\left(\frac{1}{2} - \frac{1}{2} s_2\right) = y_4y_5(1-s_2)
\]

\[
\frac{dy_5}{dm} = 2y_2y_5\left(-\frac{1}{2}\right) = -y_2y_5
\]

After calculating the elementary changes of frequencies after every possible pairing event it suffices to add them to obtain the modification in the frequencies introduced into the pool by one element (dm) of mating rounds. Integration of the complete set of differential equations gives the frequency of each molecular type as a function of the input fraction, the number of mating rounds and the probabilities of genetic exchanges.

2) **Computer analysis of the experimental data and the fitting of parameters**

Using all the available markers, associated in all the possible configurations i.e. resistant markers in cis or in trans, a great number of crosses (ca 500) has been analysed. Two, three and four point crosses have been performed using a number of strains differing both in their nuclear genetic background and in their experimental conditions. The experimental data are given in Coen et al. (1970), Boltonin et al. (1971), Avner et al. (1973), Wolf, Dujon and Slonimski (1973), Netter et al. (1974); Coen et al. (in preparation), Dujon (in preparation). The data have been introduced into a computer and tested to see whether they are compatible with the model. The details of the computer calculation will be given elsewhere (Dujon, in prep.). The experimental data are compatible with the model and permit the estimation of the parameters giving the best fit. The parameters fitted from the complete set of experimental data are given in Figure 3a, 3b, 4a and 4b. They are used to draw the theoretical lines presented in these figures.

2.1) **Variations in the input as an experimental tool for testing the model:** In bacteriophage crosses the variations in the multiplicity of infection have been used as a tool to unravel the genetic mechanisms (Visconti and Delbrück
FIGURES 3a and 3b.—Output fraction of a given allele as a function of its input fraction. Homosexual crosses: Figure 3a; Heterosexual crosses: Figure 3b. In both figures the experimental data are compared with the theoretical predictions of the model.

1) **Experimental:**

Each point represents the results of one individual cross.
- ○ crosses involving C and O
- ● crosses involving C and P
- ◇ crosses involving C, O and P
- ◆ UV irradiated crosses involving C and O

Ordinates: output fraction of the $C^a$ allele (Figure 3a); output fraction of the $C^+$ allele (Figure 3b).

Abscissae: output fraction of the $O^a$ or $P^a$ alleles (Figure 3a); output fraction of the $O^+$ or $P^+$ alleles (Figure 3b).

2) **Theoretical:**

The lines relate the output fraction (ordinates) of the $C$ allele located in a polar region to its input fraction (abscissae). They are drawn according to the model which assumes that (1) the output fraction of alleles located in nonpolar regions, such as O or P, is an adequate estimate of the input fraction of mitochondrial DNA molecules as a whole and thereby of the input fraction of the $C$ allele; (2) in homosexual crosses the output fraction of the $C$ allele is equal to its input fraction; (3) in heterosexual crosses the output fraction of the $C$ allele is related to the input fraction by the formula:

$$C^+ = w \left( \frac{e^m}{1 - w + we^m} \right)^s$$

where $C^+$ is the output fraction of the $C+$ allele
- $w$ is the input fraction of the $C^+$ allele
- $s$ is the probability of gene conversion $C^- \rightarrow C^+$
- $m$ is the average number of mating rounds

The line was drawn using the best estimates of the parameters:
- $s = 0.99$ and $m = 3.7$. 
Figures 4a and 4b—Recombinant frequencies as a function of input fraction. Homosexual crosses: Figure 4a; Heterosexual crosses: Figure 4b. In both figures the experimental data are compared with the theoretical predictions of the model.

1) Experimental:

Each point represents the results of one individual cross.
- □ crosses involving C and O
- ● crosses involving C and P
- ◊ crosses involving C, O and P
- ★ UV irradiated crosses involving C and O

Ordinates: sum of frequencies of both types of recombinants between C and O

Abscissae: output fraction of the O or P alleles (Figure 4a)
- Output fraction of the O or P alleles (Figure 4b).

2) Theoretical:

The lines relate the total frequencies of both types of recombinants (ordinates) between the C allele located in a polar region and the O allele located in a nonpolar region to the input fraction (abscissae). They are drawn according to the model which assumes that (1) the input fraction of alleles located in nonpolar regions, such as O or P, is an adequate estimate of the input fraction of mit-DNA molecules as a whole and thereby of the input fraction of both the C and O alleles; (2) in homosexual crosses the frequency of recombinants is related to the input fraction by the formula:

\[ R_{CO} = 2w (1 - w) (1 - e^{-k'm}) \]

where
- \( R_{CO} \) is the sum of frequencies of both types of recombinants between C and O
- \( w \) is the input fraction of the C and O alleles
- \( k' \) is a factor related to the probabilities of genetic exchange between C and O (cf. Dujon et al. in prep.)
- \( m \) is the average number of mating rounds

(3) in heterosexual crosses the frequency of recombinants as a function of the input fraction can be calculated by computer integrations of a complete set of differential equations (see text and Dujon et al. in prep.). The line was drawn using the best estimates of the parameters

\( s = 0.99 \) and \( m = 3.7 \)
1953). In an analogous manner we have adopted the variations of the input of mitochondrial genomes as an experimental approach. We cannot, of course, modify this input with a pipette as is the case for bacteriophages. We can, however, take advantage of the fact that nuclear genes and experimental conditions modify the input fraction from almost 0 to almost 1. The mechanisms which underly this variations of input are unknown but in spite of our ignorance the variation can be used as a tool amenable to experimentation.

Other parameters such as the average number of mating rounds or the probability of genetic exchanges are not as yet experimentally accessible. Fitting is performed assuming that these parameters either do not change from cross to cross or that their variations are relatively small with respect to the experimentally accessible variation in the input fraction. For these reasons we shall present the output of single alleles or the frequencies of recombinant types as functions of the input fraction. Each figure represents the complete set of experimental data corresponding to the example chosen together with the theoretical curve drawn from the parameters fitted by the computer.

2.2) Output of single alleles as a function of input: The model predicts that in homosexual crosses the output of every allele should be proportional to its input and therefore that the output fraction of all alleles at different loci of the same molecule which originated from the same parent should be identical. Figure 3a show that this is true. Whatever is the value of output fraction, the output of the C allele is identical to that of the O allele. The linear regression of the experimental data is in a good agreement with the theory (correlation coefficient $= 0.98$). Therefore the model fully explains one important feature of multifactorial crosses—the coordinate output of alleles.

The model predicts that in heterosexual crosses the output of alleles located in nonpolar regions such as O and P is an adequate estimate of the input fraction of mit-DNA molecules and therefore of the input fraction of C. The formula relating the output fraction of C to its input is given in the legend of Figure 3. Figure 3b shows that the experimental data are in agreement with the theoretical curve. The scattering of the experimental points is probably due on the one hand to the sampling error on the colonies counted and on the other hand to the fact that slight variations of the parameters $m$ and $s$ (which are assumed not to vary) can occur from cross to cross and that UV irradiation, in addition to its main effect on the input, may have secondary effects on other cellular processes.

In conclusion, the relationship between the output fraction of a marker such as C and its input is fundamentally different in homosexual and in heterosexual crosses. This difference exist for all the markers located in the polar region but no such difference exists for markers in nonpolar regions.

2.3) Recombination between two markers as a function of input: The frequency of recombinants between two markers varies from cross to cross and the model predicts that this variation should depend mainly upon the input. The computation of frequency of recombinants between two markers is more complex than that of the output of single alleles since the probabilities of conversion of each marker as well as their physical distances have to be taken into account.
The examples presented below are chosen as being among the simplest cases for computation, the two markers being genetically unlinked.

Figure 4a shows the total frequency of both types of recombinants between the \( C \) and \( O \) markers plotted as a function of the input fraction in homosexual crosses. The model predicts that, in the case of homosexual crosses, the frequency of recombinants should be a parabolic function of the input. The maximum frequency of recombinants is predicted for an equal input of the two parents while the frequency of recombinants should be smaller when the input is biased. No recombinants are formed for inputs of either 0 or 1. Although the scatter of the experimental data is considerable, the results are compatible with the theoretical parabole drawn.

Figure 4b gives the total frequency of both types of recombinants as a function of input between the same two markers as in Figure 4a, but for heterosexual crosses. The model predicts that the frequency of recombinants should be maximal for an input \( \text{biased in favor of the } \omega^- \text{ parent} \) (input fraction of \( \omega^+ \) molecules \( \approx 0.15 \)). The frequency of recombinants decreases rapidly for input fractions of \( \omega^+ \) molecules smaller than 0.15 while it decreases slowly for input fractions of \( \omega^+ \) molecules higher than 0.15. It is important to stress that the model explains the excess of recombinants in heterosexual crosses as compared to homosexual crosses. It explains also the large variations in recombinant frequencies observed from cross to cross or after UV irradiation. Above all the model predicts that the frequency of recombinants in certain heterosexual crosses may reach values as high as 75\%, that is there may be more recombinants than parental genomes in the progeny of a mitochondrial cross. Representation of the sum of both types of recombinants is quite similar to the representation of the major recombinant only, since the frequency of the minor recombinant type is ca. 100 times lower than that of the major recombinant type (cf. Table 1). It is a prediction of the model that the frequency of the minor recombinant should be maximal for input fraction of \( \omega^- \) genomes greater than 0.15 and consequently that the polarity of recombination should show a slight dependence on the input fraction.

2.4) \textbf{Numerical examples of two three-point crosses:} Finally, the predictions of the model and the experimental data can be compared using two examples of three point crosses, one homosexual and one heterosexual presented in Table 1. Calculations of the frequency of the eight cellular type in each cross were done using estimates of the parameters adjusted to give the best fit for the overall set of crosses. Comparisons can be made not only between the number of colonies of each type found against that predicted but also by comparing recombinant frequencies and polarity between two markers. It can be seen that the model quantitatively predicts the actual data.

\textbf{DISCUSSION}

This article outlines a model which is the first attempt at presenting a coherent and overall picture of multifactorial mitochondrial crosses in yeast. It coordinates the considerable amount of experimental data already accumulated and draws a parallel between mitochondrial genetics and bacteriophage genetics. The model
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has two aspects, one purely formal, the other having a greater bearing on the biological phenomena responsible for mitochondrial genetics.

Formal aspects of the model

The formal treatment, as do all formal treatments, suffers from two major difficulties. Although satisfactory in the sense that it leads to a quantitative agreement between the experimental data and the formal parameters it generates, it could be wrong because some of the basic biological assumptions (for instance, the idea of a panmictic pool of molecules) could be incorrect. Second, it could be just tautological. The danger of tautology increases as the number of parameters increases and a model which requires an introduction of a new parameter every time a new experimental result is obtained is, of course, not a fruitful one. It is our feeling that the model is not entirely tautological, because the estimation of parameters such as \( s \) (probability of gene conversion for one allele) and \( m \) (average number of mating rounds) can be derived from different sets of experimental data, one from the output of alleles as a function of input (see Figure 3) and the other from the frequency of recombinants as a function of input (see Figure 4). The two estimations are in agreement. Furthermore, these parameters are sufficient to account for other results such as three or four factor crosses.

Biological aspects of the model

There are two biologically important ideas inherent in the model. The first is that mitochondrial DNA molecules form a panmictic pool characterized by a variable input fraction and undertaking several random-in-time mating rounds. The second is that the elementary acts of recombination are not reciprocal.

The most direct argument in favor of the idea of a pool is the coordinate output, which describes two types of experimental results. The first type arises from the fact that in studying a great number of crosses which differ in the nuclear genetic backgrounds of the parents or in the experimental conditions one finds a continuous variation of the output from almost 0 to almost 1 for the same mitochondrial allele in the population. The second arises from the fact that this variation is strictly coordinated between all the six loci \( R_e, R_{II}, R_{III}, O_1, O_{II}, P_1 \). This second result strongly argues in favor of the idea that these six loci are carried by the same molecule of mit-DNA. This idea is further strengthened by the fact that extrachromosomal genetic determinants such as the one conferring resistance to Trialkyl Tin (\( T^R \)) (Lancashire and Griffiths 1971) do not exhibit a coordinate output with the six loci \( R_e, R_{II}, R_{III}, O_1, O_{II}, P_1 \). Therefore they are probably not located on the same molecule as the other six loci (W. E. Lancashire, in prep.). The question arises whether the mitochondrial genome is composed of two distinct molecules, one carrying the six loci presently known and the other carrying \( T^R \) determinants or is composed of only one type of molecule, the \( T^R \) determinants being carried by a molecule not located in the mitochondria. Other arguments in favour of a panmictic pool have been listed previously and are: the fact that the limit frequency of recombinants is much less than 50%; the occur-
rence of a general positive coincidence, and the relation between the frequency of recombinants and the input fraction. Furthermore, the fact that, after $\rho^+ \to \rho^-$ mutagenesis, clones issued from individual mutated cells contain several distinct mitochondrial genotypes (cf. Deutsch et al. 1974) is also an argument in favour of a pool composed of numerous genetically competent molecules in each cell.

Variations of the input to this pool may be due to a number of mechanisms. The simplest one is the quantity of mit-DNA per cell or per strain. Although this mechanism may explain some of the variation, it is doubtful however that it could be the major explanation as it is not very probable that wild-type cells will differ by a ten-fold factor in their quantity of mit-DNA. It seems more probable that nuclear genes or different physiological conditions prior to crossing may influence some properties of mit-DNA molecules such as their entrance into the mating pool. Several hypotheses can be advanced: compartmentalization due to barriers resulting from the existence of mitochondria themselves; chemical tagging of mit-DNA molecules by methylating enzymes or other enzymes leading to discrimination between molecules coming from the two parents.

It is also possible that biological mechanisms which are responsible for variation in the input may be equally responsible for diminishing the degree of panmixie of the pool. Panmixie is of course limited at the cellular level because each cell contains a finite amount of mit-DNA molecules. This limitation could be of no importance since each cell contains a relatively great number of molecules (50 to 100). Limitations at the subcellular level could, on the contrary, be very important but it is not certain whether they exist. If each mitochondrion contains a very small number of molecules and if fusion and exchanges between mitochondria are rare, this would considerably diminish the panmixie. But it is not clear at all how many mitochondria are present in a yeast cell, some workers being in favor of a great number of small mitochondria (Avers et al. 1964) while others have reported that only one giant branched mitochondrion exists in each cell (Hoffmann and Avers 1973). It is possible, that both points of view are simultaneously right and wrong in the sense that mitochondria do not exist as static entities but rather as a dynamic syncytium which continuously fuses and separates, more property called a chondriome (Guillermond 1934) (in the case of Euglena see also Calvayrac, Butow and Lefort-Tran 1972). If mitochondria constitute a dynamic system of continuously fusing and separating membrane vesicles, mit-DNA molecules would have a good chance to pair with each other during the mean time of one cellular generation.

The most direct argument in favor of something fundamentally nonreciprocal in the recombination process is the existence of the polarity of recombination related to the mitochondrial locus $\omega$. Although mechanisms other than the one proposed could have been envisaged such as preferential replication or destruction of one genome relative to the other, the advantage of the nonreciprocity of elementary acts of recombination are two-fold. It leads to an estimation of a similar number of mating rounds for all types of crosses and all segments of mit-DNA. It relates the recombination process between point mutations in a wild-type yeast (i.e. grande) to the phenomenon of petite induction, to suppressiveness and to
the known changes in the structure of mit-DNA in petites. It is well established now that petites result from important deletion and numerous repetitions of genes within mit-DNA molecules (cf. Faye et al. 1973). A mechanism of recombination which is essentially non-reciprocal is more prone to lead to the formation of repetitive DNA than a fundamentally reciprocal mechanism.

Chloroplasts of Chlamydomonas represent a classical example of extrachromosomal inheritance (Sager 1972). The genetic features of the recombination and segregation of the plastidial genomes have been, until recently, generally interpreted not on the basis of a pool of molecules but on the basis of two copies of chloroplastic genomes genetically competent per organelle (Sager 1972), although it has been shown that the amount and kinetic complexity of chloroplast DNA does not correspond to only two copies per organelle but rather to twenty (Chiang and Suedoka 1967 and Wells and Sager 1971). It will be of interest to see to what extent the contradiction between these two models (a mating pool of many copies versus a fixed diploid number of copies) is fundamental or apparent. It seems to us that many aspects of the chloroplast genetics like the uniparental inheritance controlled by the nuclear gene mt+/mt−, variation with UV as a function of irradiation and even the characteristic frequencies of certain types of genetic exchanges could also be accounted for by a panmictic pool of molecules (cf. also Gillham, Boynton and Lee 1974) undergoing non-reciprocal recombination.

This model could not have been proposed without the experimental results of our colleagues: P. Avner, M. Boloitin-Fukuhara, D. Coen, J. Deutsch, A. Kruszewksa, W. Lancashire, G. Michaelis, P. Netter, E. Petrochilo, K. Wolf and the Students of the IIIe Cycle de Génétique Approfondie. All of them have freely communicated their unpublished results to us and have contributed to many discussions during the course of the preparation of this model. The excellent technical help of MMES D. Bataille and I. Laporte is highly appreciated. The authors are greatly indebted to all these collaborators and wish to express their gratitude to them.

The authors take pleasure in thanking Dr. A. Henaut for his friendly discussions, suggestions and criticisms, J. Gabarro for designing the computer programs and Dr. P. Avner for looking through the English version of our manuscript.

This manuscript could not have been completed without the excellent help of MMES B. Duchaussoy and M. Even. This work has been supported by a grant ATP Différenciation cellulaire n° 4304 from the C.N.R.S.

LITERATURE CITED


