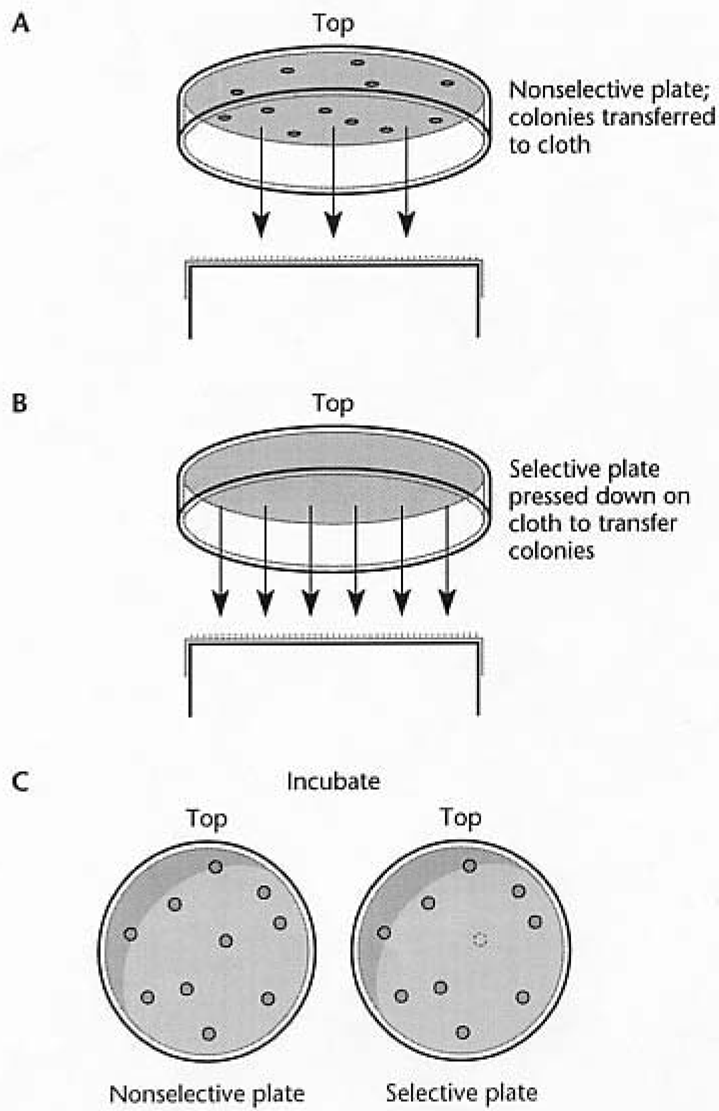
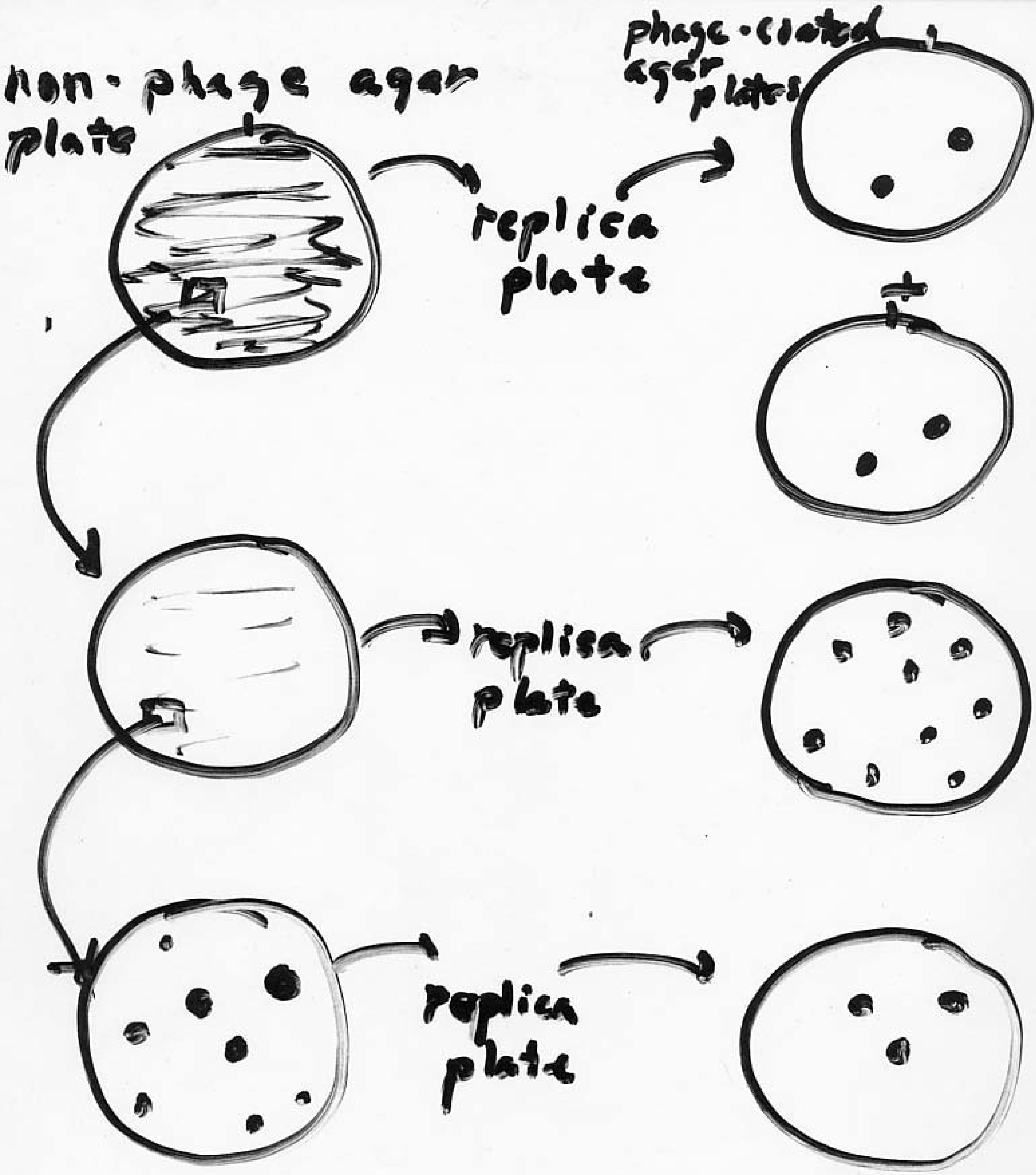
Figure 3.22


A
Top
Nonselective plate; colonies transferred to cloth
B
Top
Selective plate pressed down on cloth to transfer colonies
C
Incubate
Top
Top
Nonselective plate
Selective plate


phage-coated agar plates
non-phage agar plate
replica plate
replica plate
replica plate

MUTATION RATE

mutation rate = α = mutation/cell/÷

= mutations/cell div

= mutations/"cell generations"*

* text: Here,

"Cell generations" = no. of cell div.

≅ no. of cells (N)

Confusion source

10^8 cells → 2×10^8 cells

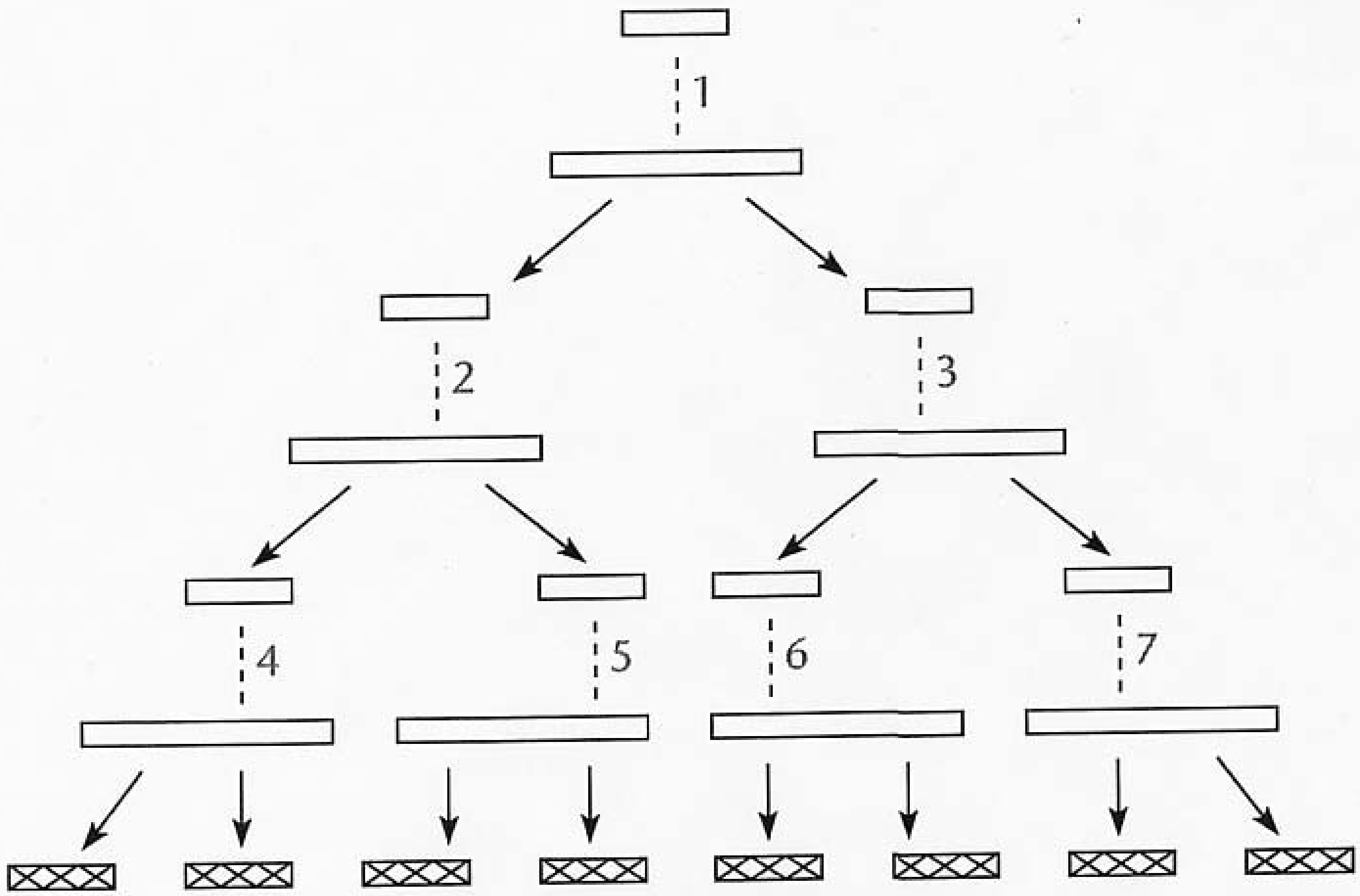
= 1 generation

= 10^8 cell divisions

Cell no 2×10^8 = 2×10^8 cell divisions

= 2×10^8 cell generations

Figure 3.7



Fluctuation Test Results:

E.g., 20 cultures; 5.6×10^8 cells/culture

11/20 cultures had no T1^r mutants, ∴ no mutations

∴, the no. of cell generations/culture is known (5.6×10^8) and the ave. no. of mutations/culture can be calculated from the Poisson equation.

Poisson Equation (Distribution)

$$P_x(r) = \frac{e^{-x} x^r}{r!}$$

$P_x(r)$ = fraction of a large no. of boxes that will contain r objects if an ave. of x objects is distributed at random among the boxes.

$0! = 1$ by defn.

$$e = \frac{1}{1!} + \frac{1}{2!} + \frac{1}{3!} \ldots = 2.718$$

$$P_i = \frac{m^i e^{-m}}{i!} \quad \text{(text)}$$

m = ave. no. of mutations/culture.

"i" = probability of having i mutations in a culture

when $i = 0$, $P_i = 0.55$

$$0.55 = \frac{m^0 e^{-m}}{0!} = \frac{1 \cdot e^{-m}}{1} = e^{-m}$$

$$m = -\ln .55 = \underline{0.59}$$

$\therefore$ mutation rate $\alpha = m/N$

$$= \frac{0.59}{5.6 \times 10^8} = 1.05 \times 10^{-9}$$

Poisson – a few general observations

For $m = 1$

$$P_1(0) = e^{-1} = 0.37$$

$$P_1(1) = \frac{e^{-1} 1^1}{1!} = 0.37$$

$$P_1(2) = \frac{e^{-1} 1^2}{2!} = 0.19$$

$$P_1(3) = \frac{e^{-1} 1^3}{3!} = 0.06$$

$$\sum_0^{\infty} P(r) = 1.$$

$$P_5(0) = \frac{e^{-5} 5^0}{0!} = .0067$$

$$= .67\%$$

Newcombe Data

$$\alpha = \frac{m_2 - m_1}{N_2 - N_1} = \frac{49 - 8}{2.8 \times 10^{9} - 2.6 \times 10^{8}}$$

$$= \frac{41}{2.54 \times 10^{9}} = 1.6 \times 10^{-8}$$

3rd procedure – <u>Chemostat</u>

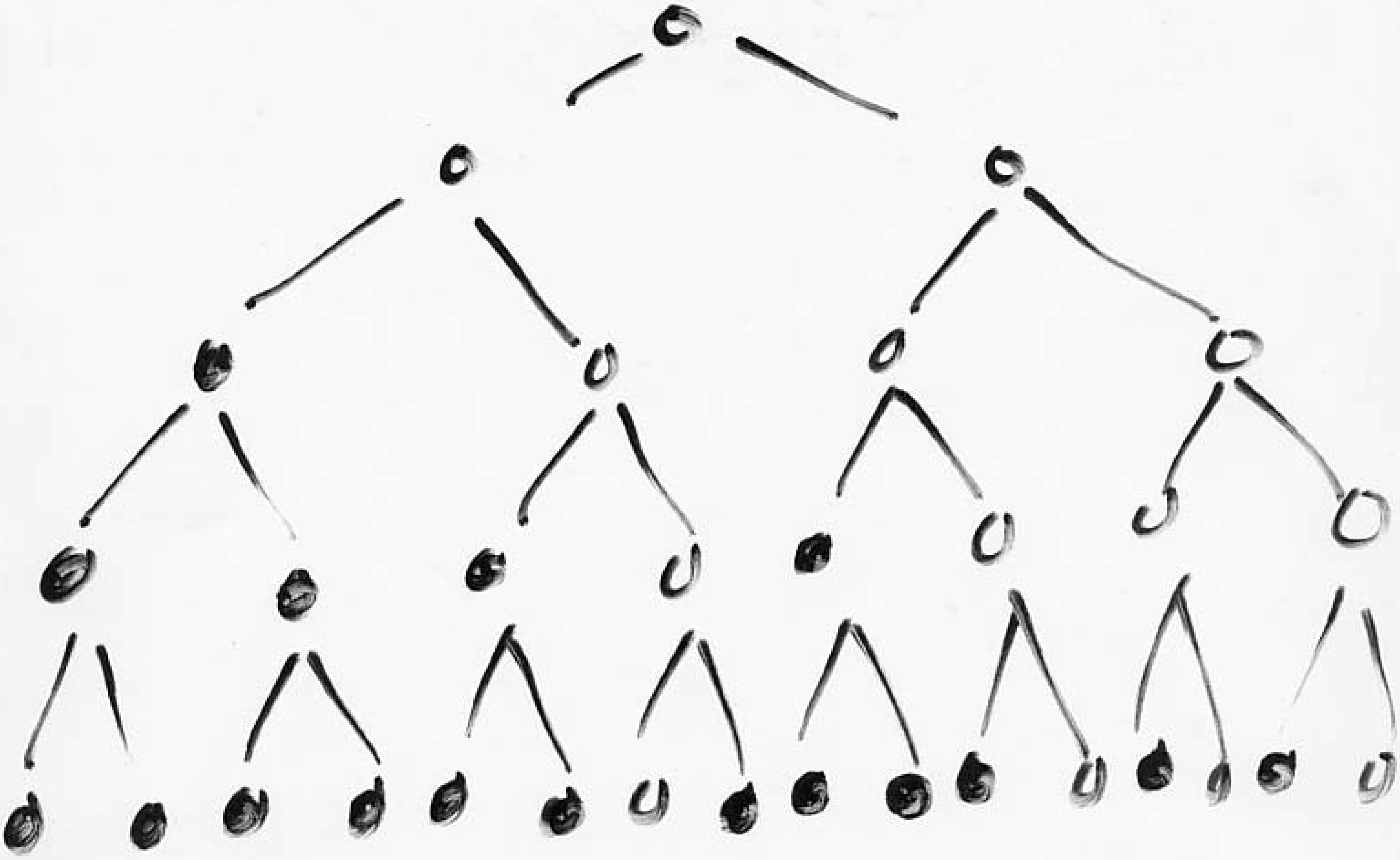
If not selected against, the total no. of mutants increases faster than the total cell population

TABLE 10

The proportion of mutants to total cells in a growing population of bacteria. It is assumed that:

1. the growth rates of mutant and wild type bacteria are identical;
2. the mutation rate (m) is very low so that the number of wild type bacteria available to generate new mutants is virtually the same as the total number;
3. the probability of back-mutation of mutant bacteria to wild type is negligible over the experimental period.

Generation (division) cycles		1	2	3	4
Total No. bacteria	$N \longrightarrow$	$2N \longrightarrow$	$4N \longrightarrow$	$8N \longrightarrow$	$16N$
No. old mutants			$4mN$	$16mN$	$48mN$
			+	+	+
No. new mutants		$2mN$	$4mN$	$8mN$	$16mN$
			$\}\ 8mN \nearrow$	$\}\ 24mN \nearrow$	$\}\ 64mN$
Proportion mutant/total bacteria		m	$2m$	$3m$	$4m$



each gen. contributes the same no. of mutants to the final population

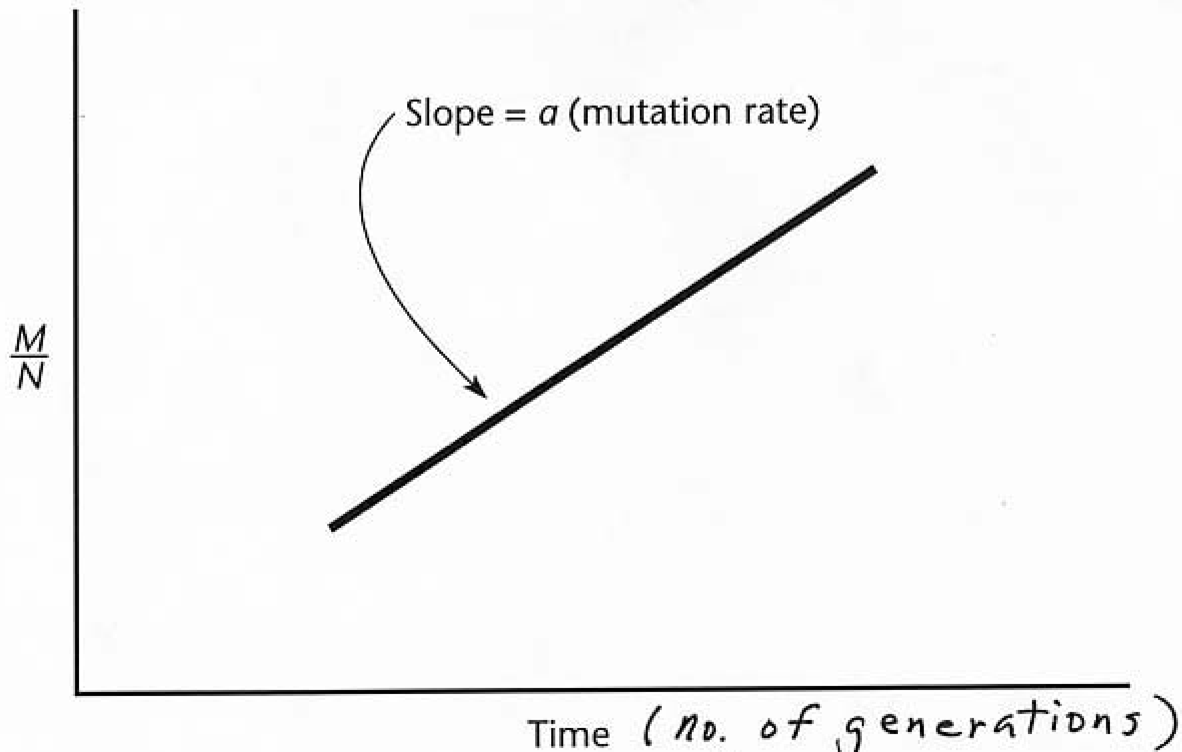
$$n = \underline{g}\,\alpha N$$

$$n = \underline{\underline{g}}\,\alpha N/2$$

$$n = \underline{\underline{g}}\,\alpha N/.693$$

where n equal no. of mutants

Figure 3.8



no. of mutants $= n = agN$

$$n/N = ag$$

$$y = mx + b$$