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The kinetics of chromosome transfer in *Escherichia coli*: a mathematical treatment

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The following picture of bacterial conjugation has been put forward in the preceding paper by de Haan & Gross (1962) on the basis of some new experimental results. When Hfr and F^- cells are mixed together they first form effective contacts. After a variable delay, chromosomal transfer is initiated in these conjugating pairs of cells. At any time after the effective contacts have been formed there is a certain probability that the pairs will separate. As a consequence of this separation either the segment of the Hfr chromosome that has been injected into the female is extracted, or the chromosome is broken at the point where it enters the F^- cell, leaving the injected fragment in the female. In their experiments de Haan & Gross limited the time in which effective contacts could be formed to 5 minutes, so producing an essentially synchronized population in which the subsequent events in the mating process could be studied.

In this paper we shall present a mathematical description of this picture of bacterial conjugation, and compare its predictions with the experimental results reported by de Haan & Gross. The following assumptions will be made in developing the mathematics. At time $t = 0$ there is a population consisting of $N(0)$ mating pairs in which no genetic transfer has taken place. The time at which transfer commences in these pairs varies from $t = 0$ to $t = \lambda$. A constant fraction p per unit time (where $p\lambda = 1$) of the $N(0)$ pairs initiates transfer throughout this period. After transfer has commenced, the Hfr chromosome is transferred at the same constant rate in all pairs. At any time after $t = 0$ each pair has a probability of separation equal to $b + c$ per unit time, where b and c are constants. The probability that this separation is accompanied by the withdrawal of the Hfr chromosome is b per unit time, while the probability that it is accompanied by the breakage of the chromosome is c per unit time. All Hfr markers which remain in the female cell have the same probability of incorporation into F^- recombinants.

On the basis of these assumptions we want to calculate the results of two types of experiment performed by de Haan & Gross. In these experiments the number of F^- recombinant cells which contain a certain marker is measured after treatment of the population at different times during growth, in one case by blending, and in the other case by the adsorption of T6 phage particles to the Hfr parents. Blending mechanically breaks the pairs and in so doing leaves the injected part of the male chromosome in the female. Treatment of the male cells with T6 stops any further transfer, but does not affect the rate, $b + c$, of the subsequent separation of pairs.

Apart from a constant factor relating to the probability of incorporation of the transferred marker into a recombinant, the blendor experiment measures at any time t the number of F^- cells which contain a particular marker for which the time of transfer is T minutes. We shall designate this number by the function $g(t,T)$, and in future refer to this marker simply as the T marker. In the T6 experiment, phage is added to the population at some time, t_1. As no further chromosome transfer occurs, the number of F^- cells which contain the T marker will decrease due to spontaneous separation of the pairs, and associated withdrawal. Again, allowing for the incorporation factor, this experiment therefore measures the number of F^- cells which still contain the T marker at time $t = \infty$. This number will be designated by $h(t_1,T)$.

The calculation of $g(t,T)$ and $h(t_1,T)$ involves the prior calculation of two further quantities, $N(t)$ and $f(t,T)$. $N(t)$ is the total number of intact mating pairs present at time t; while $f(t,T)$ is the number of intact mating pairs in which the T marker has entered the F^- cells. We shall now calculate these four functions $N(t)$, $f(t,T)$, $g(t,T)$ and $h(t_1,T)$ in turn.

The calculation of each of the three functions which involve T falls naturally into three parts depending on whether: (i) $t < T$, when the T marker has not entered any F^- cells, so the three functions are all zero, (ii) $T < t < T+\lambda$, when the marker is being transferred into new F^- cells, and (iii) $t > T+\lambda$, when all transfer of the T marker has been completed.

Calculation of the total number of mating pairs [N(t)]

As the original $N(0)$ pairs separate with a constant probability $b+c$ per unit time, it follows that for all values of t,

$$N(t) = N(0)\,e^{-(b+c)t}. \tag{1}$$

Calculation of the number of mating pairs which contain the T *marker* [f(t,T)]

(a) $T < t < T+\lambda$

In the time interval δt, $f(t,T)$ will increase by the number of pairs in which the T marker has just been injected into F^- cells. This number equals $pN(t)\,\delta t$. During the same time δt, $f(t,T)$ will decrease because of the separation of pairs in which the F^- cells previously contained the T marker. The number of such pairs which separate in time δt is just $(b+c)\,f(t,T)\,\delta t$. We have, therefore

$$f(t+\delta t,T) = f(t,T)+pN(t)\,\delta t-(b+c)\,f(t,T)\,\delta t,$$

and substituting from (1) and writing $f(t,T)$ simply as f, this gives the equation:

$$\frac{df}{dt}+(b+c)\,f = pN(0)\,e^{-(b+c)t}, \tag{2}$$

which is to be solved with the boundary condition

$$f = 0 \quad \text{when} \quad t = T.$$

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By straightforward methods this solution is found to be

$$f(t,T) = pN(0)(t-T)e^{-(b+c)t}. \tag{3}$$

(b) $t > T+\lambda$

As no further injection of the T marker is possible, equation (2) reduces to

$$\frac{df}{dt}+(b+c)f = 0.$$

The solution to this equation, which is continuous with (3), is

$$f(t,T) = N(0)e^{-(b+c)t}.$$

The complete solution for $f(t,T)$ is therefore:

$$\left.\begin{array}{ll} f(t,T) = 0 & t < T \\ pN(0)(t-T)e^{-(b+c)t} & T < t < T+\lambda \\ N(0)e^{-(b+c)t} & t > T+\lambda \end{array}\right\} \tag{4}$$

Calculation of the theoretical curves for the blendor experiment [g(t,T)]

(a) $T < t < T+\lambda$

In the interval δt, $g(t,T)$ will increase due to those mating pairs in which the T marker has just been injected into F^- cells, which again is $pN(t)\delta t$. It will decrease by the number of those pairs, in which the F^- cells contain the T marker, that separate *and withdraw* the male chromosome. This number equals $bf(t,T)\delta t$, therefore

$$g(t+\delta t,T) = g(t,T)+pN(t)-bf(t,T),$$

which leads to the equation:

$$\frac{dg}{dt}+bf = pN, \tag{5}$$

with the condition $g = 0$ when $t = T$. On substituting for N and f from (1) and (4) and solving this equation, one finds:

$$g(t,T) = \frac{pN(0)}{b+c}\left\{\left[b(t-T)-\frac{c}{b+c}\right]e^{-(b+c)t}+\frac{c}{b+c}e^{-(b+c)T}\right\} \tag{6}$$

(b) $t > T+\lambda$

Here equation (5) reduces to

$$\frac{dg}{dt}+bf = 0,$$

for which the solution, continuous with (6), is

$$g(t,T) = \frac{b}{b+c}N(0)e^{-(b+c)t}+\frac{pN(0)c}{(b+c)^2}e^{-(b+c)T}[1-e^{-(b+c)\lambda}]. \tag{7}$$

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Calculation of the theoretical curves for the T6 experiment [h(t_1,T)]

(a) $T < t < T+\lambda$

At the time t_1, when phage T6 is added to the culture, there are, from (4), $f(t_1,T) = pN(O)(t_1-T)e^{-(b+c)t_1}$ mating pairs in which the T marker has entered the F^- cells. As the adsorption of T6 stops all further transfer, this number falls off, due to separation, according to the equation $\frac{df}{dt} = -(b+c)f$, which has the appropriate solution

$$f(t_1,t,T) = pN(0)(t_1-T)e^{-(b+c)t}.$$

Corresponding to this value for f the number of F^- cells which contain the T marker is now given by the equation $\frac{dg}{dt}+bf=0$, in conjunction with the boundary condition (obtained from (6)) that

$$g(t_1,T) = \frac{pN(0)}{b+c}\left\{\left[b(t_1-T)-\frac{c}{b+c}\right]e^{-(b+c)t_1}+\frac{c}{b+c}e^{-(b+c)T}\right\}.$$

The solution to this equation is

$$g(t_1,t,T) = \frac{b}{b+c}pN(0)(t_1-T)e^{-(b+c)t}+\frac{pN(0)c}{(b+c)^2}[e^{-(b+c)T}-e^{-(b+c)t_1}].$$

As $h(t_1,T)$ is equal to $g(t_1,\infty,T)$, we find finally:

$$h(t_1,T) = \frac{pN(0)c}{(b+c)^2}[e^{-(b+c)T}-e^{-(b+c)t_1}].$$

(b) $t > T+\lambda$

Here $h(t_1,T)$ is constant for all values of t_1 and is just equal to the value of $g(t,T)$ given by (7) as $t\to\infty$.

Results for the blendor and T6 experiments

The preceding results for g and f are collected below, where for convenience the time variable t has been replaced by $t' = t-T$, so that t' refers to the time after which the transfer of the T marker commences.

$$\left.\begin{aligned} g(t',T) &= \frac{pN(0)}{b+c}e^{-(b+c)T}\left\{\left[bt'-\frac{c}{b+c}\right]e^{-(b+c)t'}+\frac{c}{b+c}\right\} && t' < \lambda \\ &= \frac{pN(0)}{b+c}e^{-(b+c)T}\left\{\lambda b e^{-(b+c)t'}+\frac{c}{b+c}[1-e^{-(b+c)\lambda}]\right\} && t' > \lambda \\ &= \frac{pN(0)c}{(b+c)^2}e^{-(b+c)T}[1-e^{-(b+c)\lambda}] && t'\to\infty \end{aligned}\right\} \quad (8)$$

$$\left.\begin{aligned} h(t_1',T) &= \frac{pN(0)c}{(b+c)^2}e^{-(b+c)T}[1-e^{-(b+c)t_1'}] && t' < \lambda \\ &= \frac{pN(0)c}{(b+c)^2}e^{-(b+c)T}[1-e^{-(b+c)\lambda}] && t' > \lambda \end{aligned}\right\} \quad (9)$$

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Comparison with experimental results

The solutions for the blendor and T6 experiments contain the unknown parameters $N(0)$, λ (or $1/p$), b and c. Of these, $N(0)$ occurs as a constant factor, so it has no effect on the shape of the curves. The other parameters must be deduced from a comparison of the predictions of the theory with the experimental results. We shall now make this comparison for the cross HfrR4 × F$^-$. De Haan & Gross have reported results for this cross when the mating pairs have been incubated in minimal medium and broth.

For convenience, the comparison will be carried out in stages:

(1) *Estimation of* b *and* c *for minimal medium and broth*

It follows from (8) that $g(t',T)$ can be expressed in the form:

$$g(t',T) = e^{-(b+c)T} \text{ (function of } t'). \tag{10}$$

Two conclusions can be drawn from this:

(*a*) The blendor curves should be identical in shape for all markers (i.e. for any value of T), and for all growth conditions.

(*b*) For any particular value of t' the percentage of recombinants obtained for two markers T_1 and T_2 should be connected by the relation

$$g(T_1)/g(T_2) = e^{-(b+c)(T_1-T_2)} \tag{11}$$

When the mating pairs were incubated in minimal medium, the results of de Haan & Gross agree with both of these predictions. The shape of the blendor curves for the three loci, proline (*pro*), TL, and methionine (*met*), were found to be similar in shape in that they all reached their maximum when $t' = 20$, and then fell to approximately half of this value by $t' = 60$. Also, the experimental results satisfy (11), for, from Table 1 of the previous paper, we find, for $t' = 20$:

$$g(TL)/g(pro) = e^{-6(b+c)} = 0{\cdot}74 \qquad \text{so} \qquad b+c = 0{\cdot}05,$$

and $$g(met)/g(TL) = e^{-17(b+c)} = 0{\cdot}44 \qquad \text{so again } b+c = 0{\cdot}05.$$

In other words, the number of recombinants found for these three markers are related in the simple exponential manner $e^{-(b+c)T}$, where $b+c$ is constant and equal to 0·05.

When transfer is allowed to proceed in broth, agreement with the experimental results is not so close. If we substitute in (11) the results from Table 1 we now obtain

$$g(TL)/g(pro) = e^{-6(b+c)} = 0{\cdot}48 \qquad \text{so} \qquad b+c = 0{\cdot}12,$$

and $$g(met)/g(TL) = e^{-17(b+c)} = 0{\cdot}043 \qquad \text{so here } b+c = 0{\cdot}19.$$

When the mating pairs are incubated in broth the value of $b+c$ is therefore not constant. The recombination values for the three markers are thus not related in a simple exponential manner, but the probability of separation of the mating pairs increases with time. However, throughout the whole course of the experiment

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the rate of separation is greater in broth than in minimal medium. It is this greater rate of separation which causes the blendor curves in broth to flatten after $t' = 20$ in contrast to the curves obtained in minimal medium. The fact that $b+c$ has a smaller value at early times is reflected in the experimental result that the earliest marker, *pro*, does have a blendor curve which shows a small but noticeable drop after it has reached its maximum, while the later markers, *TL* and *met*, have blendor curves which are quite flat.

(2) *Estimation of* λ *during growth in minimal medium and broth*

By straightforward differentiation of (8) it follows that, quite irrespective of the value of λ, the blendor curves will have a maximum value when $t' = 1/b$. This is the time when the loss of zygotes due to extraction of the Hfr chromosome just balances the increase in zygotes due to transfer of the T marker into further F^- cells.

For minimal medium it has been shown above that $b+c = 0{\cdot}05$. The earliest time at which this maximum could occur is thus $t' = 20$, in which case $c = 0$. However, if $c = 0$, that is if all separation of mating pairs is accompanied by withdrawal, then eventually all Hfr markers will be lost from the F^- cells, and the blendor curves would approach zero. As this does not occur, c cannot be zero, so the maximum value of the blendor curves due to this cause must occur later than $t' = 20$. Experimentally, however, the blendor curves do have their maximum at $t' = 20$, so the reason for this must depend on the fact that further injection of the T marker ceases at this time. In other words, during transfer in minimal medium the extent of the delay in the initiation of transfer in the Hfr cells is 20 minutes.

During transfer in broth, the theoretical equations do not strictly apply, for, as was pointed out above, $b+c$ increases slightly with time. However, some information about the possible limits of $b+c$ can be obtained by putting $b+c = 0{\cdot}15$, which is approximately the average of the two values found above. Experimentally, the blendor curves again reach their maximum when $t' = 20$. It is therefore possible that in broth $b = 0{\cdot}05$ and that the maximum is due to the balancing of extractions with new transfer of the T marker. However, it appears more likely that, just as when transfer occurs in minimal medium, the maximum at $t' = 20$ is due to λ being equal to 20. We shall assume this is so in the following discussion, in which case it follows that for transfer in broth $b \leqslant 0{\cdot}05$.

(3) *Estimation of* b *and* c *in minimal medium and in broth*

For transfer in minimal medium the blendor curves eventually level off to a value which is approximately equal to half their maximum, so we can put $g(20)/g(\infty) = 2/1$. Substituting into equations (8), and putting $\lambda = 20$, one finds the solutions $b = 0{\cdot}032$, $c = 0{\cdot}018$. Separations which lead to extractions of the Hfr chromosome are therefore more common during transfer in minimal medium than are those which lead to breaks.

If, during transfer in broth, we put $b+c = 0{\cdot}15$ and $\lambda = 20$, and give b its maximum possible value of $0{\cdot}05$, then one finds $g(20)/g(\infty) = 1{\cdot}07$. With these values a

drop in the blendor curves of only about 7% would therefore be predicted. For smaller values of b, this drop becomes even less, until at the limit, when $b = 0$, there is no drop at all. As the experimental results for the *TL* and *met* loci are quite compatible with a drop of 7% or less, it can be seen that any solutions for which $b < 0{\cdot}05$, and so $c > 0{\cdot}1$, are satisfactory. When $b + c < 0{\cdot}15$, as is the case at early times during transfer in broth, then the predicted drop is larger and would be noticeable, as is found experimentally with the proline marker which is transferred earliest of the three markers studied.

A comparison of these results for broth and minimal medium shows that the value of c is much greater during transfer in broth than it is in minimal medium; it is this variation which alters the shape of the blendor curves under the two growth conditions. The value of b is critical in determining the shape of the blendor curve in minimal medium, but, within limits, can have quite a wide variation in broth. In fact, it is consistent with the experimental data to assume that b has the same value 0·032 both in broth and minimal medium, and the whole effect of the growth conditions is to vary the probability, c, at which chromosomal transfer is interrupted by the breakage of the Hfr chromosome. The theoretical curves which follow from this assumption are presented below.

(4) *Shape of theoretical blendor and T6 curves*

In Fig. 1*a* are shown the theoretical curves for the *TL* marker when transfer is arrested in one case by blending, and in the other by the addition of phage T6. These curves are calculated from equations (8) and (9) with the following values of the parameters:

minimal medium: $b = 0{\cdot}032$, $c = 0{\cdot}018$, $\lambda = 20$, $p = 0{\cdot}05$, $T = 10$
broth: $b = 0{\cdot}032$, $c = 0{\cdot}118$, $\lambda = 20$, $p = 0{\cdot}05$, $T = 10$

These theoretical curves are to be compared with the experimental curves of de Haan & Gross, some of which are reproduced in Fig. 1*b*. In making this comparison it should be borne in mind that there is a certain variability in the experimental results. As pointed out by de Haan & Gross, there is usually a drop of about 1/2 observed in the blendor curves obtained in minimal medium, and it is on this value that the calculations were based. However, in some experiments this drop can be as little as 1/3 (as in the experiment of Fig. 1*b*), or as great as 2/3.

From Fig. 1 it can be seen that the main features of the theoretical and experimental curves are quite similar. The main difference is that the theoretical curves for transfer in minimal medium show a greater discontinuity at their maximum than is apparent in the experimental curves. There are two factors which could account for this discrepancy. Firstly, in the experiments of de Haan & Gross effective contacts are formed over a period of 5 minutes, while in the calculations it has been assumed they all occur simultaneously. Secondly, it has been assumed in the calculations that the Hfr chromosome is being transferred at exactly the same rate in all zygotes. Some small variation in this rate is almost certain to occur. Both these factors would tend to introduce a degree of asynchrony into the

transfer which would manifest itself by a less pronounced discontinuity at the maximum value of the blendor curves.

By a straightforward extension of the theory the first of these factors, the variation of 5 minutes in the time of formation of effective contacts, can be taken

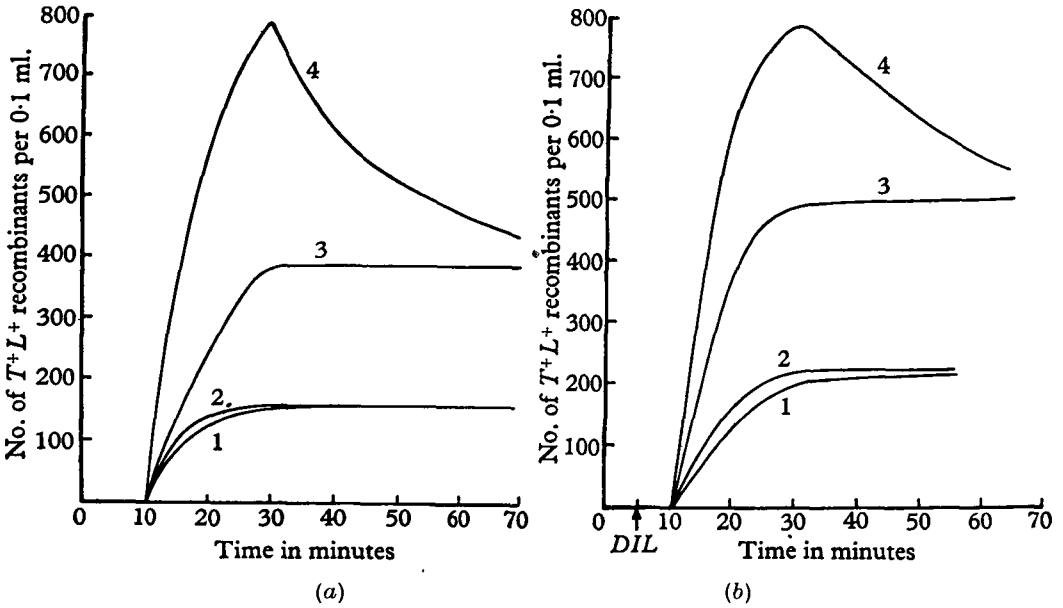

Fig. 1. A comparison of the theoretical (1*a*) and experimental (1*b*) transfer curves for the *TL* marker obtained either by blending or by treatment with the phage T6. The numbers on the curves refer to the following growth conditions: (1) broth, T6 treated; (2) broth, blended; (3) minimal medium, T6 treated; (4) minimal medium, blended. The value of $N(0)$ used in calculating the curves in Fig. 1*a* has been chosen so that the maximum of the curve 4 has the same value as that of the maximum of the analogous experimental curve in Fig. 1*b*.

into account. When this is done, it is found that the value of the three parameters, λ, b and c, which best fit the experimental results are slightly altered from those deduced above, but the main conclusions are identical with those derived from the simple treatment developed here.

CONCLUSIONS

The following conclusions (most of which have been pointed out by de Haan & Gross) can be drawn from the comparison of the experimental results with the predictions of the theory:

1. The ideas of de Haan & Gross concerning chromosome breakage and withdrawal are sufficient to explain the experimental data.

2. For the cross HfrR4 × F^- the simple assumptions of the mathematical theory outlined in the beginning of this paper lead to predictions which fit closely with the experimental results when the mating pairs are incubated in minimal medium.

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3. The simple assumptions do not strictly hold when the mating pairs are incubated in broth, as it must be assumed that the probability with which the mating pairs separate is not constant, but increases during conjugation.

4. The difference between the shape of the blendor curves when transfer occurs either in broth or in minimal medium depends on the fact that during incubation in broth there is a much greater probability that transfer will be spontaneously arrested due to breakage of the Hfr chromosome.

5. The period of 20 minutes which elapses before the blendor curves for any marker reach their maximum is determined by a delay of up to approximately 20 minutes in the initiation of chromosome transfer by some Hfr cells. Furthermore, the extent of this delay seems unaffected by whether the mating pairs are incubated in broth or in minimal medium.

SUMMARY

A mathematical treatment has been presented of the ideas of de Haan & Gross concerning transfer delay and chromosomal withdrawal during conjugation in *Escherichia coli*. The calculations involve three parameters: (i) the maximum delay, λ, which can occur between the formation of effective contact in mating pairs and the initiation of chromosomal transfer, (ii) the probability, b, that mating pairs separate with the withdrawal of that segment of the Hfr chromosome which has entered the female cell, and (iii) the probability, c, that mating pairs separate with the breakage of the Hfr chromosome at the point where it enters the female cell, leaving the injected fragment in the female.

A comparison of the theory with the experimental results of de Haan & Gross obtained when chromosomal transfer occurs either in minimal medium or in broth shows good agreement under the following conditions:

(i) the value of λ is the same under both growth conditions,
(ii) the value of b is the same under both growth conditions,
(iii) the value of c is much greater during transfer in broth than it is in minimal medium.

I should like to thank Dr P. de Haan and Mr J. D. Gross for the many enjoyable discussions concerning their experimental results, which were the stimulus for these calculations.

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