ABSTRACT

The idea that large DNA molecules adopt a stretched conformation as they pass through gels suggests a simple mechanism for the separation of DNA by crossed field electrophoresis: at each change in field direction a DNA molecule takes off in the new direction of the field by a movement which is led by what was formerly its back end. The effect of this ratcheting motion is to subtract from the DNA molecule's forward movement, at each step, an amount which is proportional to its length. We find that this model explains most of the features of the separation, and we describe experiments, using a novel electrophoresis apparatus, which support the model. The apparatus turns the gel between two preset orientations in a uniform electric field at preset time intervals. This separation method has the practical advantage over some others that the DNA molecules follow straight tracks. A further advantage is that the parameters which determine the separation are readily predicted from the simple theory describing their motion.

INTRODUCTION

Schwartz and Cantor have devised a way of separating large DNA molecules that is likely to have a wide range of applications. Their method uses a novel form of gel electrophoresis in which the DNA travels through a concentrated agarose gel under the influence of two electric fields. The two field angles are set near to perpendicular, are non-uniform in field strength, and are alternately switched or pulsed. It is generally assumed that in high gel concentrations and at high voltages, long DNA molecules must be stretched out along the direction of the field in order to penetrate the pores of the gel. Schwartz and Cantor explain the separation they achieve by alternating field directions as resulting from the need for DNA molecules to reorient themselves in the gel in order to travel in a direction at right angles to the axis along which they are stretched. The longer the DNA molecule, the longer the time taken to find the new orientation and the more the molecule is held back in the gel. Inhomogeneity in the field is thought to be necessary to turn the molecule in the new direction of movement.

This paper presents an alternative explanation for the separation. We believe that DNA
molecules retain their extended conformation between pulses and that on changing the direction of the field, molecules move in the new direction led by what was the trailing end during the previous pulse. As a molecule moves forward in the new direction, it must turn itself through the pore occupied by this end, and as the position of this end depends on the length of the molecule, long molecules will be held back more than short ones.

This simple idea makes a number of testable predictions, and we describe experiments that confirm most of them.

MATERIALS AND METHODS
Design of the apparatus. To test the model we needed an electrophoresis apparatus which could alternate the direction of the field with respect to the gel, and which had uniform voltage gradient across the gel. These requirements are difficult to meet by switching electrodes, and we used a conventional horizontal electrophoresis tank, altering the field angle between pulses by rotating the gel (Fig 1). The machine we built is a simple box holding a pair of electrodes, with a turntable for the gel in the centre. The turntable is driven by a DC motor, coupled through a magnetic drive so that the base of the box is sealed. On receiving a pulse from the timer, the motor drives the turntable round until it is stopped by a microswitch. At the next pulse, a relay reverses the polarity of the supply to the motor which is driven in the opposite direction until it meets a second microswitch. The microswitches are thrown by pins set in the spindle of the magnetic drive, the pin positions can be altered to alter the angle of turn. The apparatus allows variation in the angle of the field to the gel, and in the voltage and duration of the DC to the electrodes. Buffer was circulated through an external cooling device which maintained a constant temperature preset to between 5° and 20°C.

The plug mould, slot former and gel casting plates were also made in our laboratory workshop. 12, 16 and 22 cm diameter gels were cast by first sticking autoclave tape around the edge of the circular glass plate, sealing the edges by trickling in some molten agarose and then, with the plate on a horizontal surface, pouring a 1.5% solution of agarose (Sigma type II, medium EEO) in 0.5 x TAE. For electrophoresis in the presence of ethidium bromide, 20 μg/ml ethidium bromide was added to the gel as well as to the electrophoresis buffer. Following electrophoresis, 10 changes of distilled water were needed over 4 days to destain this gel for photography. All other gels were routinely stained in 0.5 mg/l ethidium bromide over 30-60 min followed by 1-2 h of destaining. Gels were photographed using a Photodyne UV transilluminator and Kodak Professional Technical Pan film through a Wratten red and blue green filter combination or interference filter with peak transmission at 590 nm and a half band width of 10 nm.
Fig. 1 Plan of the electrophoresis apparatus: The box is 27 cm square, with electrodes at each end. The circular gel (22 cm diameter) is held on a turntable driven from below. The two positions of the sample wells during the run are shown. The change in field angle experienced by the DNA molecules is $110^\circ$.

DNA samples. Yeast chromosome markers (Saccharomyces cerevisiae X2180-1B, a gift from Dr. Jill Ogden) were made by embedding cells in agarose as described by Schwartz and Cantor\(^1\) and by Carle and Olson\(^2\). Briefly, cells were grown in YPD (2% glucose, 2% bactopeptone and 1% yeast extract) at 30°C to O.D. of 0.36 at 600 nm. Cells were harvested and suspended in 1.2 M sorbitol, 20 mM EDTA, 14 mM $\beta$-mercaptoethanol to a concentration of $5 \times 10^8$ cells/ml. Cells were warmed up to 37°C and mixed 1:1 with a 1% solution of LGT agarose (low gelling temperature SeaPlaque agarose, FMC Bioproducts) made up in the same sorbitol, EDTA, $\beta$-mercaptoethanol mix and kept at 37°C. The cells and agarose mixture was poured in a plug mould and allowed to set. The plugs were removed into a solution of 1.2 M sorbitol + 20 mM EDTA, 14 mM $\beta$-mercaptoethanol, 10 mM tris- HCl pH 7.5 and 1.0 mg/ml Zymolase-20T. They were incubated at 37°C for 2 h followed by lysis in 1% lithium dodecyl sulphate, 100 mM EDTA, 10 mM Tris- HCl pH 8.0 at 37°C over 1 h. The solution was replaced with fresh solution and incubation continued at 37°C overnight. These plugs could now be stored in this solution at room temperature. For storage at 4°C, the solution was replaced with one containing lauryl sarcosine instead of lithium dodecyl sulphate.

Lambda oligomers were made using $\lambda$cI857Sam7 DNA (monomer = 48.5 kb). Virions were made by the induction method\(^3\) and the oligomers made either directly by embedding
virions in agarose or by preparing DNA and oligomerising in liquid. Virions were embedded in 0.5% LGT agarose at a DNA concentration of 30 μg DNA/ml. A mould was used to make the plugs and these were then treated with 0.5M EDTA, 1% lauryl sarcosine, 0.5-1.0 mg/ml Proteinase-K at 50°C overnight. Plugs were washed several times in 2 × SSC (1 × SSC = 150 mM NaCl, 15 mM sodium citrate pH 7.0). They were then equilibrated in 2 × SSC, 10 mM Tris-HCl pH 7.5, 10 mM EDTA and left at room temperature for 1-2 weeks for oligomerisation. The plugs can be stored in this solution at 4°C. For making oligomers in solution, the virions were first processed to make lambda DNA using gentle pipetting and mixing to minimise shear damage. Oligomerisation was carried out by adjusting the concentration of DNA to 200 μg/ml in 2 × SSC, 3% ficoll. This mix was incubated at 37°C for 30min followed by incubation at room temperature overnight. These oligomers are stable at 4°C for at least one year. Detailed descriptions of these and other pulsed field gel electrophoresis protocols can be obtained directly from R.A.

THE UNDERLYING THEORY

The shape and dimensions of DNA in solution and in gels. The shape adopted by DNA in gels is not known. Yanagida et al have shown that, in free solution, long DNA molecules alternate rapidly between different conformations, which they describe as flexible rods, compact spheres and ellipsoids. The ellipsoid is the shape adopted for most of the time. However, they also showed that when DNA solutions flow over a surface, the DNA molecules are stretched into filaments which lie in the direction of flow. There is no similar direct analysis of the shape adopted by DNA molecules during gel electrophoresis, but the fact that extremely long DNA molecules can penetrate concentrated gels suggests the molecules must be extended as they pass through the gel. Other observations, in particular the greater mobility of linear as compared with circular molecules, suggest that the movement of linear DNA molecules through the pores of a gel is most readily started by an end. It has been suggested then, that as a DNA molecule is forced through the pores of a gel, one end of the molecule leads the movement in the direction parallel to the field; movement of this end is always in a forwards direction. The other end points backwards along the same axis, and between the two ends the chain winds through the pores of the gel along the path opened up by the leading end. Comparison of the average pore size of a 1.5% agarose gel, which is of the order of one tenth of a micron, with the contour length of phage lambda DNA, which is about 16 microns, supports this view. An important consequence of this progressive form of movement is that there can be no back tracking; the leading end can never cross the path of the molecule.
trailing behind. Viewed from the side the molecule will be extended in such a way that no segment crosses any other.

The average distance between the ends of a DNA molecule in the random coil configuration is approximately proportional to the square root of its contour length. For a molecule which is extended in the way suggested above, the end to end distance will be shorter than the contour length but proportional to it (J.K.Elder, pers. comm.). Thus for any partially extended molecule, the relationship between the average end to end distance and contour length must be

\[ h_{av} = kL^n \quad (0<k<1, 0.5<n<1) \]  

(1)

where \( h_{av} \) is the average end to end distance, and \( L \) is the contour length of the chain. Assuming the model to be correct, our data, shown below, indicate that \( n = 1 \), suggesting that the DNA molecule is extended.

Others have assumed that the DNA molecule undergoes major reorientation between successive pulses. It is an important assumption of the model we propose that the extended orientation, once adopted by the DNA, is retained when the current is switched off. Yanagida et al. measured the diffusion rate of T4 DNA in free solution as 1.2 \( \mu \)m/sec. This is fast, and if it were the rate of diffusion in gels, it would allow for sufficient translational movement of a DNA molecule to displace it by its own dimension in the time taken for a single pulse. Our theory could not be valid if diffusion in gels were so fast. However, there are reasons for believing that the rate of diffusion in gels is much slower, the most cogent of which is that bands of high molecular weight DNA in agarose gels remain sharp for several days after electrophoresis. The rate of thermal motion of large DNA molecules in gels must therefore be several orders of magnitude lower than the rate imposed by electrophoresis, and we conclude that in the few seconds between pulses, there cannot be time for large DNA molecules to undergo major reorientation. (Yanagida et al. also show that molecules in free solution alternate rapidly between conformations which differ in end to end length by a large factor. At present there are no equivalent observations on molecules in gels, but we assume again that trapping the DNA in the gel stabilises an extended conformation.)

**The path of an extended chain in crossed field gel electrophoresis.** Above a certain size all DNA molecules passing through a gel by conventional electrophoresis, move at the same rate. We assume that they are extended and that their front ends penetrate the gel together and move together. In this case their back ends will trail behind at distances which depend on the lengths of the molecules. When the current is switched off, the molecules will be held in this extended state.
If the direction of the field is changed in such a way as to push the DNA molecule side-ways, the molecules can only begin to move in the new direction by leading off with one of its ends, or by forming a new "end" by kinking; movement led by a kink requires more work and probably occurs less often than movement led by a true end.

If the new field direction is at right angles to the old direction, the new leading end can be either end; if it is less than 90° the new end is likely to be the old front end. But if it is greater than 90° the new front end is likely to be the old back end because leading with the old front end would turn the molecule through a sharper angle, which would require more work. As the back ends of the molecules were left at different positions in the previous pulse, the starting point for this movement is different for molecules of different length (Fig 2).

Thus the first prediction of the model is that if the angle between the fields is less than 90°, DNA molecules will behave as they do in a gel that is run conventionally i.e. with no turning, whereas if the angle is greater than 90°, they will be held back in the gel at each turn by a distance that is proportional to their length.

It follows from this that the separation between molecules at the end of the run will be proportional to the difference in their lengths.

It also follows that the separation will be proportional to the total number of pulses, and up to a certain limit discussed later, the separation will be independent of pulse length. The effect of pulse length on separation observed by Schwartz and Cantor and by Carle and Olson is really an effect of the number of pulses, as their experiments were run for the same total time and therefore the number of pulses was inversely proportional to pulse length. We describe experiments which show the effects of pulse length more directly.

The model may be expressed more precisely as follows:

The distance moved by the front end of any molecule along the direction of the field is

\[ d = vt \]  

where \( v \) is the velocity of DNA in conventional gel electrophoresis under the same conditions and \( t \) is the duration of the pulse.

The distance moved by the front end relative to the axis of the gel is

\[ m_i = d \cos \theta \]  

where \( \theta \) is the angle of the field to the mid-line of the gel.

The distance moved by the back end of the molecule is

\[ m_i = (d - h_i) \cos \theta \]
where $h_i$ is the apparent end to end length of the molecule, related to its contour length by

$$h_i = kL_i^n \quad (0<k<1, \ 0.5<n<1)$$

In one pulse, the distance separating the back ends of two molecules $i$ and $j$ will be

$$m_i - m_j = -(h_i - h_j) \cos \theta \quad (5)$$

After $N$ pulses, the distance moved by a molecule will be

$$M_i = Nm_i = N(d - h_i) \cos \theta = (D - Nh_i) \cos \theta \quad (6)$$

where $D$ is the distance moved by DNA in conventional electrophoresis under the same conditions and for the same total time.

The separation between molecules $i$ and $j$ will be

$$M_i - M_j = N(m_i - m_j) = -N(h_i - h_j) \cos \theta \quad (7)$$

Separation is proportional to the number of pulses $N$.

For a total run time $T$, $N = T/t$.

Hence, separation is inversely proportional to pulse length.

**Size limit for separation.** During a pulse, the distance moved by the back end of a molecule is $d - h$. If $h$ is greater than $d$, the back end will not progress past the position occupied by the front end on the previous pulse. Separation will extend up to molecules whose apparent length is equal to $d$. Since $d$ is proportional to the pulse length, the size limit for separation will increase in proportion to the pulse length, an effect noted by Schwartz and Cantor$^1$ and by Carle and Olson$^2$.

**RESULTS AND DISCUSSION**

**Experimental evidence for the model.**

**Switching field angle in a uniform field.** Many of the predictions of our model are met by the published observations of Schwartz and Cantor$^1$ and Carle and Olson$^2$. They have shown that separation is inversely proportional to the duration of the pulse, and that the limit for separation increases with increasing pulse time. We have confirmed these observations, but the critical tests of our model are first, that separation occurs at obtuse but not at acute field angles, and second, that there is no requirement for a non-uniform field, which is an important component of the model proposed by Schwartz and Cantor. We built the device described in Materials and Methods, which produces alternating field angles within a uniform field. In early models, the turntable was square, held in a cradle, and driven from above. We found that this design produced enough non-uniformity in the field to distort the tracks considerably.
Fig. 2 Model for separation mechanism: Movement of molecules during two sequential pulses and the separation by size resulting from changing the leading end from the front to the back of the molecules. Equations 1 to 7 are based on this model. The DNA molecule, represented here as a rod, is probably a partially folded chain.

(a) Two molecules, i and j, at the end of the first pulse, their leading ends are together leaving their back ends separated by $h_i - h_j$. Projected onto the midline, the separation becomes $(h_i - h_j) \cos \theta$; ($\theta > 45^\circ$).

(b) At the start of the second pulse, i and j turn round their trailing ends: o and o show positions of turning points.

(c) Positions of i and j after the second pulse. d is the distance moved by the front end of any molecule during one complete pulse along the direction of the field.

The problems were removed by driving a circular turntable from below.

**Effect of field angle.** The model we propose suggests that separation will occur if the angle between the two fields is greater than $90^\circ$ and not if it is acute. Two electrophoresis separations were carried out under identical conditions of voltage, buffer and temperature: in one run the gel was stationary with its mid line parallel to the field; in another with the gel swung through an angle of $80^\circ$ at intervals of 8 sec. The total time of the run was adjusted to take account of the effect of field angle.

We were surprised to find some separation of lambda DNA oligomers in the stationary gel (Fig 3a). C.Tyler-Smith suggested that this might be due to larger molecules running faster than small, as he has shown that this does happen under some conditions of electrophoresis, in unpublished experiments. However, under our experimental conditions, the smaller ran faster than the larger molecules. This was shown by running lambda DNA monomer alongside the
Fig. 3 Effect of field angle: The first panel shows λ-oligomers, λ-monomer and λ-monomer + λ DNA restricted with HindIII, run on a stationary gel with no turn or change of polarity (conventional high voltage horizontal gel electrophoresis). Note that under these high voltage conditions, the monomer is separated from the dimer. The second panel shows the result of turning the gel through an acute angle, 80°.

oligomers, and also by 2-D electrophoresis with the second dimension run under crossed field conditions: the bands that moved fastest in the stationary gel also moved fastest in crossed field electrophoresis (data not shown).

In support of the model, we find that at an angle of 80°, the separation is similar to that in the stationary gel (Fig 3b), whereas, with an obtuse field angle, there is good separation of the lambda DNA oligomers under similar running conditions (see below). The mobility of the monomer in the stationary gel was 4.3 μm/sec, the higher molecular weight molecules ranged from 4.0 to 4.2 μm/sec. In the gel switched through 80°, the corresponding mobilities, measured along the centre line were 2.8 and 2.4 to 2.55 μm/sec. Adjusted for the field angle, the
Fig. 4 Effect of reversing polarity before acute turn: Conditions were as for Fig 3b, except that the polarity of the current was reversed before the gel was turned, and current was maintained during the turn. The two tracks are λ-oligomers and λ-DNA digested with R.HindIII.

net rate of movement during the pulse is 3.6 μm/sec for the monomer, and 3.1 to 3.3 μm/sec for the oligomers. These measurements suggest that turning the gel through an acute angle has an effect on mobilities, but the effect is the same for molecules of all sizes. By contrast, turning the gel through an obtuse angle causes a size dependent deceleration of the molecules in the oligomeric series.

Effect of reversing the current before changing the field angle. If turning the field through an obtuse angle does have the effect of swapping the leading end from the front to the back of the molecule, the same effect might be achieved by reversing the polarity of the current, in
order to reverse the direction of the molecule, and then turning the gel, with the current still switched on, through an acute angle. This regime did indeed give separation of the lambda oligomers (Fig 4), lending further support to the model.

**Relationship between DNA length and mobility.** A striking feature of the band pattern formed by the oligomeric series of lambda DNA is the uniformity of spacing between members of the series (Fig 5a). The model predicts that a molecule will be held back at each step by a distance proportional to a simple function of its length (Fig 2): the uniformity of spacing of the oligomeric series over a considerable range (Fig 5b & c) suggests that the relationship between the end to end distance of the DNA and its contour length is close to proportionality, that is that \( n \) is approximately equal to one in equation (1). The non-uniform spacing above a certain size is a function of pulse length and is explained below.

**Effect of pulse length.** Schwartz and Cantor\(^1\) showed that with short pulses, molecules in the low size range are well separated and molecules above a certain size all travel together. Increasing the pulse time has two effects: the spacing between bands of DNA in the low molecular weight range is decreased, and large molecules which are not separated at short pulse times are now separated. This same behaviour was found using our apparatus, and both effects are explained by the model. For those molecules which are separated, the separation is a result of pulling molecules back at each pulse, by an amount that is proportional to length (Equation 4). The total amount by which a molecule is held back depends the number of pulses produced in the run, and as a longer pulse time produces fewer pulses in the same total run time the effect of increasing the pulse length is to decrease the separation.

Our model requires that separation is proportional to the number of pulses (Equation 7). That this is so can also be seen from the data in Fig 5, which shows that separation is approximately proportional to the reciprocal of pulse time. The model also suggests that the size limit for separation will be proportional to pulse length, as this limit is equal to the distance moved by any DNA molecule during the pulse. The largest lambda oligomer in the uniformly separated series is proportional to pulse time (Fig 5c).

**Molecules above a certain size are not separated.** This is expected from the model: to make net forward movement during a pulse the back end of a molecule must pass the point occupied by its front end at the start of the pulse (Fig 2). Molecules above a certain size will be longer than the distance covered during the pulse and on the turn will "hang" in the same position. Decreasing the pulse length brings smaller molecules into this class. The simple model, which assumes that DNA is a flexible rod cannot explain the fact that long molecules do not "hang" at the point of application, but penetrate the gel to a considerable distance. A
Fig. 5 Effect of pulse time: (a) Yeast chromosomes in agarose plugs and liquid λ oligomers run on 1.5% agarose gels. Electrophoresis was in 0.5×TAE at 150V and 20°C. Pulse times were, from left to right, 10 sec, 20 sec, 40 sec, 60 sec, 90 sec and 120 sec. The resolution of DNA molecules in the range >1000 Kbp, can be improved by reducing the electrophoretic voltage and increasing the pulse time. (b) Mobilities of λ oligomer series obtained by scanning gel photographs on a digitising densitometer, band centres were found by fitting Gaussian curves. Each plot has a linear part but, note the downward curve in the 10 to 60 sec pulses. This represents the widening of the separation in the high molecular weight range as discussed in the text. (c) A plot of the differences in mobilities which emphasises the uniform separation of the λ oligomeric series and the wider separation of the high molecular weight range seen in (a) and (b).

A more complex model which allows variation in the end to end length of the DNA chain predicts this penetration.

Between those molecules that are not separated and those that are separated by a distance proportional to their length, there is a run of oligomers which are spaced wider than the uniform spacing lower in the size range. Spacing is uniform up to a point, above which it increases for a few steps before it is compressed up to the point where no separation is observed (Fig 5a and 5c). This pattern can be also explained by assuming that the DNA
chains are not rigid, and the end to end distance varies from one pulse to another: molecules which are close in length to the distance travelled in a pulse will sometimes be too extended to pass the turning point and therefore on some pulses will make no net progress, decreasing their mobility beyond what is predicted by the model. Computer simulations predict the observed patterns quite closely (E.M.S. and J.K. Elder work in progress).

**Estimation of end to end length of lambda DNA.** The apparent end to end distance of lambda DNA during electrophoresis can be calculated in two different ways. First, from equation (7) above, \( N \) and \( \theta \) are parameters set for the run. \( M_i \) and \( M_j \) can be measured for all adjacent pairs in the oligomeric series of lambda DNA and

\[
h_{\text{monomer}} = h_i - h_j \tag{8}
\]

Secondly, the limit for the separation occurs when \( h > d \), the distance moved on each pulse, thus

\[
h = d / n_{\text{max}} \tag{9}
\]

where \( n_{\text{max}} \) is the highest \( n \)-mer separated.

\( h_\lambda \) was calculated in both ways for various conditions of separation (Table 1). The values calculated by the two methods are in remarkably good agreement, suggesting that the model is correct in principle. The data also show that the apparent end to end length increases with the voltage gradient, which is in keeping with the basic assumption of the model, as it is to be expected that DNA molecules would be elongated by increasing the voltage gradient.

The measurements suggest an end to end length of about 5.5 microns during electrophoresis at 8 V/cm in a 1.5% agarose gel, a value which is about one third of the contour length measured in the electron microscope by the surface spreading technique, and two to three times the long axis of the ellipsoid seen in free solution by fluorescence microscopy.

**The Effects of Ethidium Bromide.** If the model is correct, mobility of DNA and the separation between molecules of different size should be affected by factors which change the end
Fig. 6 Effect of Ethidium bromide:

2μg of λ oligomers (loaded in solution) were run on two 1.5% agarose gels. The left lane is from a gel run in the presence of 20μg/ml ethidium bromide, the right lane is from a gel run without ethidium bromide. Electrophoresis conditions for both gels were identical i.e. 150V in 0.5 x TAE, 60sec pulses, 20°C and a total run time of 33h.

to end length of DNA molecules. Acridine dyes and drugs such as ethidium bromide increase the length of DNA by intercalating between adjacent base pairs\textsuperscript{10}. A separation of the lambda oligomer series was carried out in the presence of EtBr at a concentration of 20μg/ml in conditions otherwise the same as those used in the previous experiment.

Ethidium bromide had three effects: it reduced the mobility of all molecules; it increased the separation between members of the series; it brought smaller molecules into the size range that was not separated (Fig 6). All of these effects are predicted from the model, as results of increasing the end to end length of the DNA chains.
Sidestepping. It is commonly found in crossed field electrophoresis, using either the Schwartz and Cantor\(^1\), or the Carle and Olson\(^2\) apparatus that the bands of DNA in the high molecular weight range lie off the tracks defined by the combined field vectors, which can often be seen as smears running from the loading slots. This is readily explained by the model as a consequence of inequality in the strength of the two fields or the duration of the two pulses. If the two are unequal, a molecule may not pass the turning point with the field in one orientation while it can when the field is applied in the other. Thus for each cycle it will not move on the weaker or shorter pulse but on the stronger or longer pulse it will make a step in the direction of that field, taking it off the track. We confirmed this interpretation by running gels in our apparatus with pulses of unequal duration in the two field orientations. It can be seen that molecules small enough to cover their length during a pulse travel along the
Fig. 8  Straight tracks maintained over a large usable area: Yeast chromosomes and λ DNA oligomers in agarose plugs, run on a 22cm diameter 1.5% agarose gel in 0.5 × TAE, 20°C. Electrophoresis was at 150V with a pulse time of 65sec and a total run time of 33h.

Lines parallel to the tracks. By contrast, molecules which are separated more than those in the uniformly spaced region are also those which step off the track (Fig 7). This result is consistent with the explanation that both effects are caused by molecules occasionally hanging around the turning point.

Limitations of the model.

Experimental results show that the model explains the main features of crossed field separations. However, the model as presented so far does not explain all features of the separation.
and in view of the assumptions made, this is not surprising. Inadequacies are most apparent at the high end of the molecular weight range; for example, the fact that very large molecules, in the size range that shows no size separation, penetrate the gel to a considerable distance, is not explained by the model. We are testing more complex models to try to find the physical basis for this observation. One possibility is that very large molecules frequently penetrate the gel led by a kink rather than an end and that the longer molecules may move through pores in a folded rather than a fully extended form especially at high voltages. Whether this explains the penetration of large molecules or not, it is of practical consequence that this effect of high voltages could explain why bands are broader in gels run at high voltages where kinking is more likely to occur, than in gels run at low voltage. If at any pulse a molecule can lead with either a kink or an end, end to end length will vary considerably from pulse to pulse and spread out the band during a run. We find that the voltage gradient must be kept below 6 V/cm to minimise broadening the bands in the molecular weight range of 500-1000 kb. Much lower voltage gradients are needed to separate molecules in the size range of chromosomes from Schizosaccharomyces pombe (unpublished results of C.Tyler-Smith).

A more serious limitation is that the model cannot explain the separations achieved by field inversion as described by Carle et al\textsuperscript{11}, the rods assumed by our model would not separate in their system, and as they speculate, the explanation of their separation is likely to be found in the flexible coil properties of the DNA molecule.

CONCLUSION

A rigorous theoretical treatment based on a more realistic description of the behaviour of DNA in gels which treats it as a flexible coil will lead to a fuller understanding of the mechanism of separation. However, there is a close fit between the predictions of the simple model, which treats DNA as a flexible rod, and experimental tests. This suggests that ratcheting provides a correct explanation for the principal mechanism of separation for crossed field gel electrophoresis, rather than time taken to reorient the molecule in the new direction of the field, as suggested by other authors\textsuperscript{1,2}. It is likely that the model explains the separation obtained in all the different apparatus designs that use crossed fields\textsuperscript{1,2,4,13}. The model has allowed us to design a novel gel electrophoresis apparatus which gives sharp resolution up to a size greater than 10\textsuperscript{6} base pairs, in tracks which are straight across and along the gel (Fig 8). This makes it easy to interpolate the positions of unknowns with those of markers, giving the accurate size estimates needed for mapping. A further advantage of this method of separation, is that it is easy to predict the effects of altering the pulse length and the run time, reducing the need for exploratory separations.
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REFERENCES