PREAGGREGATIVE CELL MOTION IN
DICTYOSTELIUM

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SUMMARY
The motions of a large number (495) of preaggregative D. discoideum NC-4 cells in sparse
fields are recorded on time-lapse film and analysed using a specially constructed computer
graphics system. All films are produced under a standard set of conditions, so that the range of
behaviours under given conditions can be characterized. The mean velocity of pre-
aggregative D. discoideum NC-4 is 7.19 \( \mu \text{m/min} \). The mean velocity time course has a significant
early peak at about 3 h. The distribution of mean velocities is fairly broad with a long high
velocity tail. A modified random walk model using the parameters diffusion constant and
 persistence time describes well the changes in cell direction with time. Persistence can be
described as an exponentially distributed memory of movement direction, with a mean of
4.89 min. High velocity cells never have long persistence times, and persistence time shows no
relationship with age. A nearest neighbour model of cell spacing shows that cells are randomly
(Poisson) distributed at low densities. Measurements of cell contacts are compared to a simple
model of contact frequency based on the kinetic theory of gases to show that cells at low
densities have an affinity for making collisions. The length of contact durations is indicative of
some mechanical adhesion between cells, and cells in contact move significantly though not
dramatically slower. A cross-correlation analysis shows that the various parameters of motion
are significantly interrelated in numerous ways. Finally mutants and strains related to D.
discoideum NC-4 exhibit a number of new behaviours, suggesting that motion is a distinctive
characteristic of cell type.

INTRODUCTION
The cellular slime moulds are organisms interesting to developmental biologists
because movement, intercellular communication, morphogenesis, and differentiation
are all exhibited in rudimentary form and are particularly amenable to experimental
study. One goal of slime mould research is to explain the multicellular behaviour in
terms of the combined behaviours of individual cells.

Much work has been devoted to understanding the development of one species of
cellular slime mould, Dictyostelium discoideum. In the laboratory, D. discoideum strain
NC-4 cells feed on bacteria, either on agar plates or in suspension culture. The cells
grow and divide until the bacteria are withdrawn (by washing and centrifugation).
If bacteria-free cells are placed on an agar plate, they move about as individuals for
about 8-12 h. At that time aggregation begins, and waves of coordinated cell move-
ment are seen propagating outward from centres. The inward movement of cells
continues until they collect in aggregates, the central part of which forms a tip. A
slug forms from the aggregate, with the tip at the anterior end, and after the slug migrates, a fruiting body, containing spores, erects.

The movement of the individual cells during the period after starvation, but before the onset of aggregation, is the focus of this study. Movement is one of the primary mechanisms used by slime mould cells during their development, and can be studied under controlled conditions. The study of preaggregative cell movement is important because it gives information about the behaviour of cells as individuals in an unper- turbed state, which can be used as a reference to which the behaviour of cells during aggregation, or the behaviour of mutants defective in movement, can be compared. The values of the principal parameters of motion, including the range and distribution of these values, are important in various kinds of modelling of slime mould behaviour. This has been particularly true in our simulation research (MacKay, 1978), where random individual cell motion must be accurately represented.

Heretofore, the work of Samuel (1961) involving *D. mucoroides* cell motion has been the only general study of a *Dictyostelium* species. For lack of any direct study, his results have frequently been quoted as applying to *D. discoideum* as well. Eckert, Warren & Rubin (1977) have reported some results on *D. discoideum* movement as part of their study of biochemical aspects of motility. These results were obtained from photographs, rather than films, and from cells grown in suspension culture rather than on agar plates, and thus are not directly comparable to the results of Samuel or of this paper.

The well known monograph of Shaffer (1962) draws on the work of Samuel and other cell studies in discussing cell motion in cellular slime moulds. At several points he makes speculations about the behaviour of preaggregative cells, calling for tests of the assumed hypotheses. Some of these points are difficult to study because analysing large numbers of cells is required.

Although time-lapse films are easy to produce, extracting the motion data from films is a tedious undertaking, especially in the quantities necessary to obtain a significant sample. Because several of the interesting motion parameters show fairly wide distributions, it is necessary to produce a large data sample to reflect accurately the behaviour of the population. We have produced such a sample by using a specially constructed interactive computer graphics system (Potel & Sayre, 1976), which has enabled us to digitize a large number of individual cell motions in each of a number of films. Additionally, the same hardware configuration with its interactive graphics capabilities has been used for the data analyses.

Because the results of this study are to provide a basis of comparison for other phases of the life cycle, other strains and species, and other sets of conditions, we feel it is necessary to find models useful for describing the important features of cell motion. One must define appropriate parameters of motion, estimate them using the data, evaluate and interpret their magnitude and study their interrelation.

We begin this study by measuring the deviation, range, and distribution of mean velocities. Then, in order to express the changes in cell direction with time, a modified random walk model (Gail & Boone, 1970) is applied.

Next, we measure the density of cells in each film, and the average cell separation.
A nearest neighbour model for describing cell spacing (Clark & Evans, 1954) provides a test for the comparative randomness, uniformity, or clumping in the spatial distribution of the cells. This model enjoys great popularity in ecology, but has not to our knowledge been previously applied to cell spacing.

We also measure the frequency and duration of contacts between cells, and compare the data to a simple model of collisions based on the kinetic theory of gases. This comparison gives information about the relative affinity of cells for making collisions and the tendency for contacting cells to adhere.

We perform a cross-correlation analysis and show, as one might expect, that the motion parameters from the various models are interrelated in numerous ways.

Finally, using this study of strain NC-4 as a basis for comparison, we describe several contrasting behaviours in related strains and mutants.

**MATERIALS AND METHODS**

*Origin and maintenance of cell stocks. Dictyostelium discoideum strain NC-4 and Dictyostelium minutum strain DC-4 were derived from stocks kindly provided by K. B. Raper. Dictyostelium discoideum strain Ax-3 was obtained originally from W. F. Loomis and provided to us by R. Clark. Strains P2 and N15F were isolated by C. Frantz in this laboratory after mutagenesis of *D. discoideum* NC-4 using the method of Yanagisawa, Loomis & Sussman (1967). P2 was isolated after treatment with picroloninic acid and N15F after treatment with nitrosoguanidine.*

Amoebae of the various strains were grown in association with *Aerobacter aerogenes* on a standard medium containing K-K phosphate buffer (KH2PO4 2.31 g/1.; K2HPO4 0.58 g/1.; MgSO4·7H2O 0.5 g/1.; dextrose (5 g/1.), peptone (5 g/1.), yeast extract (0.5 g/1.), and 2% Difco Bacto-agar (Bonner, 1967). Plates were incubated for 24-48 h at 22 °C.

*Preparation and filming of cells.* For purposes of filming, amoebae still in growth phase were washed from the plates and suspended in PAD diluting fluid (KCl 1.5 g/1.; MgCl2 0.5 g/1.; streptomycin sulphate 0.5 g/1.; phosphate buffer pH 6) (Sussman, 1966). The cells were centrifuged 3-4 times for 2 min each at 600 g. The bacterial-free cell suspension was then spread evenly on agar (2 % purified agar in K-K2 phosphate buffer poured to a thickness of 0.5 cm in 3.5-cm diameter Petri dishes) to give cell densities between 10^3 and 10^7 cells/cm^2. The time at which cells were placed on these plates was considered zero age. This time was 10-15 min after the beginning of centrifugation. Every film covered the time from 0.5 to 11 h after plating. At these low densities, there was no visual evidence of aggregation by the end of any of the films analysed.

The fields of cells were filmed (by T. Hunt and C. Frantz) using a Bolex 16-mm film camera attached to a Nikon microscope and a Nikon CFMA Autotimer. During filming a ring of moist filter paper was used to regulate humidity, and in no case was there a standing layer of fluid on the agar. All films were shot at 4 frames/min. Various magnifications were used giving frame widths of 0.6-2.1 mm for the films of *D. discoideum* NC-4, and up to 7.5 mm for the fast moving *D. discoideum* mutant P2. A haemocytometer grid was filmed at the same magnification before the cells were filmed for purposes of spatial calibration.

*Data collection.* A total of 20 films including 666 cells were digitized (by the authors, J. Irwin and C. Frantz), among these 14 of *D. discoideum* NC-4 (495 cells), 2 each of *D. discoideum* Ax-3 (54 cells) and *D. discoideum* mutant P2 (67 cells), and 1 each of *D. minutum* (40 cells) and *D. discoideum* mutant N15F (10 cells).

The films were digitized using the Galatea film analysis system (Potel & Sayre, 1976). A film was projected onto a screen using a stop-motion, variable speed projector (L-W International Data Analyzer Model 224A, Mark IV), resulting in an 80 × 110 cm image. The projector was run at 2-6 frames per s, depending on cell velocity. The analyst indicated x, y positions in the film (e.g. cell locations) with an acoustic digitizing pen (Scientific Accessories Corp. Graf/Pen GP-2). Successive pen coordinates corresponded to an increment of approximately 0.5 μm in the film. These x, y coordinates, along with the time (the film frame number), were sent to the...
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computer (DEC PDP-11/40 GT 44) where they were stored on disks. While the data were being entered, the system produced a corresponding animated graphics image which was projected onto the film image and synchronized with it for feedback and comparison, thus providing a check on the accuracy of the digitization.

Four types of information were digitized in these films: (1) cells were randomly selected and each one's position was followed in every frame for the length of the film or until the cell left the field of view; this cell track information was used in all the calculations involving speed and direction of cell motion; (2) the position of every cell in a particular frame was marked and this location information was used to calculate density and cell spacing; (3) the position and time of all contact events between a particular cell chosen at random and any others were noted for purposes of contact frequency and duration calculations; and (4) a combination of (1) and (3), a cell's position was followed in every frame and simultaneously a separate mark was made in frames in which there was a contact event. This information was used in calculations of cell speeds during and not during contact.

Data analysis. All subsequent analysis of these data was carried out with the aid of the same computer used for data entry. Where appropriate, the techniques used are described in the results section. Standard statistical methods used include; normal deviate test, t-test both paired and unpaired, curve fitting using chi-square minimization, chi-square tests of histogram distributions, linear correlation, and autocovariance (Bevington, 1969; Meyer, 1975).

In all statistical tests, the null hypothesis was tested, so that the probabilities given are the chance that the deviation or correlation observed could arise in a random sample. Small enough probabilities indicate that deviations or correlations are significant, large enough probabilities indicate they are not significant.

RESULTS AND DISCUSSION

Mean velocity

An analysis of cell motion seeks to summarize the behaviours measured in a population using a few well selected parameters. The most fundamental among these is mean velocity (speed) \( V \). (For list of symbols used in text, see p. 308.) Preaggregative \( D. discoideum \) NC-4 cells under our standard conditions had an overall population mean velocity of 7.19 \( \mu \)m/min. This value was obtained from 495 individual cell tracks by breaking each into 15-min (60-frame) intervals (5704 total), computing a mean velocity for each interval, and averaging these. The overall mean velocity value of 7.19 \( \mu \)m/min has a standard error of 0.198 \( \mu \)m/min based on 495 cells. By way of contrast, the velocity during \( D. discoideum \) aggregation steps has been estimated as 12 \( \mu \)m/min (Cohen & Robertson, 1971b).

A plot of population mean velocity versus age is shown in Fig. 1. The general shape of the mean velocity time course, particularly the early high velocity peak, is roughly similar to that produced by Samuel (1961) for \( D. mucoroides \). The high-velocity peak occurs at the same time (2–3 h) as the significant increase of actin production in \( D. discoideum \) Ax-3 (Tuchman, Alton & Lodish, 1974; Alton & Lodish, 1977). Eckert et al. (1977) also observed a correlation between high-velocity peaks (7–9 \( \mu \)m/min) and high actin content for \( D. discoideum \) NC-4 cells taken from suspension culture. One of their peaks occurred after 20–24 h in suspension (a period they call feeding) and the other after 32–34 h in suspension (a period they call early aggregation), but because their cells were taken from suspension, these times are difficult to compare with our velocity peak or Tuchman et al.'s peak of actin synthesis.

The overall value of mean velocity given by Samuel (1961) for \( D. mucoroides \) under
conditions comparable to ours is $7.58 \pm 0.157 \mu m/min$ for 74 cells. This does not differ significantly from our value by a normal deviate test ($Z = 1.54$, $P = 0.12$, two-tailed).

Fig. 1. Mean velocity time course. Mean velocity in $\mu m/min$ for all cells vs. age in min after plating. Each point is for a 60-min centred interval. Points are separated by 3 min. Bars show $\pm$ standard error of mean velocity at representative points. A mean velocity difference of $0.84 \mu m/min$ represents the $1\%$ significance level for a single-tailed normal deviate test.

Table 1. Deviation of mean velocity

<table>
<thead>
<tr>
<th>$T$</th>
<th>obs</th>
<th>$\sigma_s$</th>
<th>$V_{max}$</th>
<th>$V_{obs}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>18153</td>
<td>4.77</td>
<td>41.6</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>8814</td>
<td>4.55</td>
<td>38.5</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>5704</td>
<td>4.41</td>
<td>31.3</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>2617</td>
<td>4.16</td>
<td>29.0</td>
<td>0.105</td>
</tr>
<tr>
<td>45</td>
<td>1600</td>
<td>3.94</td>
<td>23.2</td>
<td>0.151</td>
</tr>
<tr>
<td>60</td>
<td>1091</td>
<td>3.80</td>
<td>21.0</td>
<td>0.132</td>
</tr>
</tbody>
</table>

Next we describe the deviation within the population about this mean using as parameters the standard deviation about the population mean and minimum and maximum velocities. Measures of dispersion from the mean must be stated with reference to the time interval over which they are measured. Table 1 gives the standard
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deviation $\sigma_v$, maximum velocity $V_{\text{max}}$, and minimum velocity $V_{\text{min}}$ for a range of interval widths from 5 to 60 min (the mean velocity $\bar{V}$ showed an insignificant change over these intervals). As would be expected $\sigma_v$ decreases, $V_{\text{max}}$ decreases, and $V_{\text{min}}$ increases with increasing interval width. The $V_{\text{min}}$ values show that in the population there are cells that hardly move for 60 min.

![Histogram of mean velocity](image)

**Fig. 2.** Histogram of mean velocity. Distribution of 5704 15-min mean velocity values for individual cells in bins 1 $\mu$m/min wide.

<table>
<thead>
<tr>
<th>Mean velocity summary statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\bar{V} = 7.19 \mu$m/min</td>
</tr>
<tr>
<td>$V_{\text{mode}} (1 \mu$m/min bins) = [5,6] $\mu$m/min</td>
</tr>
<tr>
<td>$V_{\text{median}} = 6.27 \mu$m/min</td>
</tr>
<tr>
<td>$V_{\text{rms}} = 8.43 \mu$m/min</td>
</tr>
<tr>
<td>$\sigma_v = 4.41 \mu$m/min</td>
</tr>
</tbody>
</table>

Skewness = +1.13 (long right tail with 5% of values exceeding 16.12).

In order to determine the distribution of cell velocities, we plot a histogram of mean velocities over 15-min intervals for the entire population (Fig. 2). Notice that this distribution is distinctly skewed to the right with a long high-velocity tail, though a significant number of zero values are also present. The overall shape of the histogram does not change significantly with different bin width. An attempt was made to fit
a number of standard probability distributions to this histogram (Brassard, Correia & Landolt, 1975). None of these resulted in a significant match, with a lognormal distribution coming the closest ($\chi^2 = 70.18$, $N = 29$). The standard distribution summary statistics are given in Table 2.

The large value of $\sigma_v$ is reflected in the width of the distribution of population 15-min velocities in Fig. 2. Looking at individual cell motions reveals that there are cells that always move slowly, cells that are always fast, and cells that move at different speeds over time, specifically many cells that stop at various times in their motion. One can produce a standard deviation of velocity that only reflects the deviation among the different cells by replacing all the 15-min velocities of each cell with that cell’s mean velocity (alternatively, one can compute the standard deviation of the cell mean velocities each weighted by the length of the cell’s time series). The resulting standard deviation is $3.56 \mu m/min$ and may be regarded as the limit of the standard deviations in Table 1 for long interval widths. This shows that a large component of the total population deviation is among cells of different speeds as opposed to being accounted for by changes of velocity in individual cells over time. The wide range of behaviours among cells is relevant to interpretation of the mean velocity time course in Fig. 1. Many cells do not follow this overall time course, and it is only after superimposing a number of individual cell time courses that the overall trend emerges.

The long high-velocity tail in Fig. 2 reflects the existence in the population of a small number of very fast cells. A separate attempt was made to digitize rapidly moving cells in order to determine the maximum cell velocity ever observed. The maximum velocity for one cell over a 15-min interval was $40.8 \mu m/min$, with a value of $37.8 \mu m/min$ for an interval of 2 h.

**Diffusion constant and persistence time**

Having described the speeds at which cells move, we desire some measure of the directions in which cells go and how these change with time. In particular we will be interested in the phenomenon of persistence (Gail & Boone, 1970; MacNab & Koshland, 1973), the tendency of a cell to keep moving in the same general direction for a period of time. We first show that *D. discoideum* cells exhibit persistence in their motion. Then we describe a random walk model for quantifying this effect and present methods for calculating its value.

It is easy to establish that there is a persistence effect in *D. discoideum* cells. For all cells the direction of motion at every point in time was computed. The change in a cell’s direction between times separated by a given interval was then computed for all times and a histogram of these angles formed, following Gail & Boone (1970). Fig. 3 shows the histograms for 3- to 45-min intervals. The bunching of values around $0^\circ$ in Fig. 3A clearly shows the tendency for cell direction not to change over 3-min intervals, a time in which the average cell has moved in excess of 2 cell diameters, a cell diameter being about $10 \mu m$ (Bonner & Frascella, 1953). However, by 45 min in Fig. 3D the histogram is indistinguishable from that drawn from a uniform distribution ($\chi^2 = 31.4$, d.f. = 34, $P = 0.59$), indicating that by this time the persistence effect is lost.
Figs. 3A and B. For legend see opposite.
Fig. 3. Histograms of change of direction in degrees between successive 1-min time intervals in 10° bins for A, t = 3 min; B, t = 15 min; C, t = 30 min; D, t = 45 min.
The motion of wandering amoebae is generally described as being 'random', that is, cells overall move in randomly chosen directions. Some effort has been made to define and test formally a random walk hypothesis for cellular motion (Gail & Boone, 1970; Peterson & Noble, 1972). Most basic random walk models describe motion as a simple diffusion process. The so-called 'Drunkard's walk' model presumes that a sequence of steps are taken each in a randomly chosen independent direction. It can be shown (Gamow, 1961) that the expected distance \( R \) away from the starting point after \( n \) discrete steps of length \( s \) is \( \sqrt{n} \), or hence the mean squared displacement \( \langle R^2 \rangle = sn \). More generally, randomly moving particles in 2 dimensions can be described as a diffusion process in continuous time \( t \), resulting in the formula

\[
\langle R^2 \rangle = 4Dt,
\]

where \( D \) is the diffusion constant after Chandrasekhar (1947). The diffusion constant is essentially a measure of how much ultimate or net progress a cell makes over time. The diffusion constant can be measured for random cell motions by calculating for each time interval \( t \) the net distances cells have gone, squaring these, taking the average, and plotting them for increasing \( t \). The resulting graph of mean squared displacement \( \langle R^2 \rangle \) vs. \( t \) should be linear with slope \( 4D \) passing through the origin.

However, the graph of this relation for real cell motions is generally not linear. Because of persistence, over a small enough time interval \( t \) a cell moves in a straight line a distance \( \sqrt{t} \), so that in this range \( \langle R^2 \rangle \) is proportional to \( t^2 \), i.e. is a parabola. Typically, plotting \( \langle R^2 \rangle \) vs. \( t \) for cell motion data results in the curve shown in Fig. 4. Gail & Boone (1970) have described the essential features of this curve using two parameters. The first of these is the (modified) diffusion constant \( D^* \), where \( 4D^* \) is the slope of the ultimately linear portion of the curve. The second parameter is called persistence time \( t^* \) and is defined by extending the linear portion of the curve to its \( x \)-intercept. Since it describes how quickly the curve changes from a parabola to a straight line, persistence time \( t^* \) provides a measure of how long cells tend to move in the same general direction before changing to a random new direction.

Gail & Boone (1970) further have derived an exact formula for the curve in Fig. 4. This requires a description of the distribution of instantaneous values of persistence exhibited during the course of a cell’s motion. A standard assumption for this purpose is an exponential distribution (Gail & Boone, 1970; Berg & Brown, 1974; Nossal & Weiss, 1974; Lovely & Dahlquist, 1975). If an exponential distribution is assumed then the graph of mean squared displacement in Fig. 4 has the equation

\[
\langle R^2 \rangle(t) = 4D^*(t-t^*(1-\exp(-t/t^*))),
\]

where the persistence time \( t^* \) obtained directly from Fig. 4 will equal the mean of the exponential distribution. The exponential distribution provides the intuitive description of persistence time in terms of a 'half life' memory of direction of motion. If one records the absolute directions in which cells are headed at given points in time, half of them will still be going within \( \pm 90^\circ \) of this direction \( t^* \) later, 25% of them \( 2t^* \) later, etc.

For every cell a plot of mean squared displacement \( \langle R^2 \rangle \) vs. \( t \) was formed and a best
Cell motion in Dictyostelium

fit of (2) to the graph obtained by chi-square minimization with respect to $D^*$ and $t^*$. In general, these fits were good enough to warrant the assumption of an exponential distribution. The mean values of $D^*$ and $t^*$ were then obtained by averaging the cell-by-cell values weighted by the length of the time series for each cell. A summary of the resulting values for pre-aggregative *D. discoideum* NC-4 appears in Table 3.

![Graph](image)

**Fig. 4.** Typical graph of mean squared displacement $x 10^3 \mu m^2$ during time intervals of duration $t$, vs. $t$ in min, each point is an increment of 15 s; has form of Eqn. 2.

<table>
<thead>
<tr>
<th>$D^*$ ($\mu m^2/min$)</th>
<th>$t^*$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>135.6</td>
</tr>
<tr>
<td>s.d.</td>
<td>99.1</td>
</tr>
<tr>
<td>Skewness</td>
<td>+1.46</td>
</tr>
<tr>
<td>5% of values exceed</td>
<td>425.0</td>
</tr>
</tbody>
</table>

An alternative method of computing the parameters $D^*$ and $t^*$ involves the use of velocity autocovariance (see Appendix). This method, which can be directly related to the method of Gail & Boone (1970), is generally easier to compute, and allows certain of the calculations to proceed without an assumption as to the underlying persistence distribution. Values of $D^*$ and $t^*$ were also produced using this method cell-by-cell.
and differed insignificantly from those produced using the method of Gail & Boone (1970), providing a useful check on our application of the modified random walk model.

This model, involving the parameters $D^*$ and $t^*$ to describe changes of direction over time, should also be able to account for velocity values (see Appendix). In particular, the quantity $S = \sqrt{(zD^*/t^*)}$ should be correlated with $V_{\text{me}}$ (by equation A.8). A scatter plot of these values computed cell-by-cell is shown in Fig. 5, and has an excellent correlation ($r = 0.939$) for such a large number of points.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{scatter_plot.png}
\caption{Scatter plot of mean velocity in $\mu m/min$ vs. $S = \sqrt{(zD^*/t^*)}$ in $\mu m/min$ for 443 cells.}
\end{figure}

The particular relationship between persistence time and velocity is of interest. For every cell with a track of at least a 30-min duration, persistence times and mean velocities were produced and a scatter plot of these values formed, Fig. 6. The 443 points have a correlation of $-0.370$, showing a significant ($P < 0.001$) negative dependence. The most striking feature of Fig. 6 is the absence of points in the upper right section of the graph. Although slow cells may have long or short persistence, fast cells never show long persistence.

It is also of interest whether there is a correlation between persistence and age, for example, with longer straight line motions later in preaggregation. Cell data were broken into 1-h time series, with persistence times computed for these, averaged for
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Each 1-h interval, and plotted versus age, Fig. 7. No trend is visually discernible. Moreover, when the 1062 individual persistence values were correlated with age, only a very small positive correlation of 0.042 was obtained, which is insignificant ($P = 0.19$) even for this large number of points. Further relationships involving persistence time will be discussed in the Sections on pp. 299 and 301.

![Graph](image)

Fig. 6. Scatter plot of persistence time in min vs. velocity in μm/min for 443 cells.

Density and cell spacing

The cell density of each film was calculated in at least 4 different frames, usually separated by 2 h. This was done by counting all cells in each of several frames of the film and dividing this number by the known film frame area. The average density for all 14 films was $1.81 \times 10^4$ cells/cm$^2$, with densities ranging from $1.76 \times 10^4$ to $9.88 \times 10^4$ cells/cm$^2$. The maximum variation observed within a single film was 30%.

The randomness of cell spacing can be measured using the same data gathered for the density studies. Clark & Evans (1954) defined the statistic mean distance between cells and their nearest neighbours and derived its expected value to be

$$r_e = 1/(2\sqrt{N})$$

assuming a random (Poisson) spatial distribution of cells with density $N$. The actual mean distance to the nearest neighbour $r_A$ can be easily computed from the data and
a ratio \( Q = \frac{r_A}{r_E} \) formed. The value of \( Q \) is therefore 1 for a random distribution of cells. Values of \( Q \) significantly greater than 1 imply more regular or uniform spacing of cells, approaching a theoretical maximum of \( 2\sqrt{2/3} = 2.1491 \) for a hexagonal (honeycomb) distribution. Values of \( Q \) significantly less than 1 imply clumping or aggregation in the cell distribution.

Clark & Evans (1954) also derived the expected standard error of \( r_E \) for a randomly distributed population. This allows \( r_A \) to be compared with \( r_E \) using a normal deviate test, resulting in the probability \( P_s \) that the spatial distribution is indistinguishable from random. The values of \( r_A \) measured in our films were significantly close to the expected values \( r_E \) for 13 of the 14 films of NC-4 cells (\( Q \) between 0.95 and 1.16, \( P_s \) between 0.08 and 0.95), thus the cell spacing in these films is random (Poisson). The exceptional film was the highest density film, which had a \( Q = 1.85 \) indicative of significantly more uniform spacing (\( P_s < 0.0001 \)). Indeed, at close packing (~10^6 cells/cm^2) the cells in a monolayer would be expected to approach the theoretical maximum value of \( Q \). Even for all 14 films the average value of \( Q \) was 1.10 with an average \( P_s \) of 0.551.

Several things may be said concerning these results. First, they verify that in none
of the NC-4 films at any age was there evidence of aggregation or clumping in the sense of a value of $Q$ significantly less than 1.

These results also show that, at low densities ($< 5 \times 10^4$ cells/cm$^2$), estimation of mean cell spacing from the value of density will be in error if such an estimation assumes uniform distribution of cells. A formula for mean intercellular distance which has been used is $2/\sqrt{(\pi N)}$ (Cohen & Robertson, 1971a; Alcantara & Monk, 1974), which is derived by assuming a uniform distribution of cells, and by approximating the area around each cell as a circle. This formula results in an overestimation by 5% of the maximum theoretical separation (based on hexagons) and an overestimation, by a factor of 2.25, of the random separations which occur at low densities.

Finally, from experiments involving the spreading of high density ($\sim 10^8$ cells/cm$^2$) drops of cells, Samuel (1961) and Keating & Bonner (1977) have postulated the existence of a negative chemotaxis or repulsion effect for *Dictyostelium*. Our spacing results suggest that this effect may be insignificant at lower densities, since cell spacing at low density is indistinguishable from random whereas a significant repulsion effect would cause cell spacing to become more uniform.

**Contacts**

Contacts between cells were measured by following 222 cells in 11 films and marking where and when a cell made and broke contact with other cells. Under the magnifications used, it is not possible to observe whether cell membranes are actually touching during contact. However, as the cells make contact, there is an obvious fusion or flowing together of the visible outlines of the cells, which may be the joining of their surrounding fluid. If 2 cells came into contact this was counted as a contact for each of them, but if a third cell contacted the pair, a contact was counted only for the third cell and not for the pair already in contact. Three of the 14 NC-4 films were not examined for contacts because they contained periods of poor focus, which although acceptable for following cell movement, were not acceptable for determining when a cell made contact. The 11 films measured for contacts had a mean cell velocity of 7.69 $\mu$m/min, compared with 7.19 $\mu$m/min for all 14 films.

From this set of measurements, it is possible to obtain information about certain aspects of cell contact. The 222 cells had 1322 periods of contact during the time they were observed. Thus, the mean contact frequency was 1.74 periods of contact/cell/h.

The number of individual contact events per cell per hour including contacts made while the cell was already in contact can be estimated by dividing the total number of contact periods by the amount of time cells were not in contact ('free time'). This value for mean contact event frequency was 2.20 contacts/cell/h.

The mean contact duration (time in continuous contact with some cell or cells) was 7.17 min/contact, but the standard deviation of contact duration was very large (8.40 min). The histogram of contact duration (Fig. 8) shows that most contacts were of short duration, but that there were a significant number of long contacts. The distribution is very highly skewed to the right with skewness = 3.28 and 5% of the values in excess of 22.9 min. The product of contact frequency (contacts/total time/
cell) and contact duration (time/contact) is the fraction of time spent in contact. For all cells, this was 20.8% of the total time.

Fig. 9 shows the time course of contact duration. This figure shows that mean contact duration increases with age for the first 8 h after starvation, but at least for the low-density fields of cells examined here does not continue to increase. The values range from 4 min between 1 and 2 h to 10 min between 7 and 8 h.

Fig. 8. Histogram of contact duration. Distribution of 1322 individual contact durations in bins 1 min wide.

Fig. 10 shows the time course of contact frequency, with the data binned in the same 1-h groups as for Fig. 9. This figure shows that after an initial peak of about 2.5 contacts/cell/h between 2 and 3 h, contact frequency decreases and remains about constant at about 1.4 contacts/cell/h. Contact event frequency (based on free time) shows the same time course with values 30% higher. Contact frequency appears to have a time course roughly similar to that of velocity (Fig. 1). In the Section on Correlations the question of whether contact frequency and velocity are directly correlated will be studied.

It has been suggested (Ramsey & Harris, 1973) that contact frequency is related to density and velocity in leucocytes. In the kinetic theory of gases (Kauzman, 1966), the expected value of contact frequency for randomly moving molecules is shown to be:

\[ \text{cf} = \sqrt{2} \frac{N}{V} d, \]
where \( d \) is molecule diameter, \( N \) is molecule density and \( \bar{V} \) is mean velocity. Intuitively, the derivation is based on the volume (area) swept out by particles over time. We will compare our measured contact frequencies against those predicted by this simple model, which assumes that the cells are oblivious to each other.

If we take this theory as a first approximation to cell contact behaviour we would at least expect that cell contact frequency would be proportional to \( NV \), and this was found to be the case (see Correlations). If the actual values of density and mean velocity for each film and a cell diameter of 10 \( \mu \text{m} \) (Bonner & Frascella, 1953) are used in equation (4), then the calculated contact frequency can be compared with that measured from films. In only 1 film out of 11 studied was the measured contact frequency lower than that predicted, and this was the film with the highest cell density. In the other 10 films the actual contact frequency was higher than predicted by factors of between 1.2 and 3. For all 11 films, the increase of actual contact frequency over that predicted is significant \( (t = 2.94, P = 0.016 \text{ by a two-tailed paired } t\text{-test}) \). The actual contact event frequency (based on free time) is even higher than the contact frequency based on total time (by 30%), and thus comparing
contact event frequency to the predicted values would only strengthen the same result. Thus, *D. discoideum* cells made more contacts than would be expected for simple collisions between randomly moving objects, suggesting that cells have a degree of affinity for each other.

Since an average cell contact lasted 7-17 min, and the average cell velocity during contact can be estimated at 6-06 μm/min for all films (see below), cells on the average moved in excess of 40 μm (4 cell diameters) during contact. This provides an indication of the degree of physical adhesion between cells, which typically appear to flow together upon making contact.

The velocities of cells before, during, and after contact were measured in each of 3 films for 81 cells making 843 contacts. These films were not unlike the other 8 films with respect to their contact frequency and contact duration, but had slightly (16%) higher velocities than the population as a whole. The results of this study of contact velocity are summarized in Table 4. Cell tracks were broken into a series of segments, a non-contact segment followed by a contact segment followed by a non-contact segment, etc. All 81 cell tracks began with a non-contact segment, but tracks ended with either a contact segment or a non-contact segment. Only those contacts which had both a pre-contact and a post-contact segment were included, which is the reason why 828 pairs are included in each test. The velocities were compared by a paired *t*-test; for example, the velocity during each of the 828 contact segments was compared...
with the velocity in the preceding non-contact segment. These comparisons show a significant decrease in cell velocity during a contact compared with both before and after a contact, but an insignificant difference in velocity from before a contact to after a contact. When the non-contact segments were broken into smaller sections, and only the 15-min section of the cell track immediately before or after a contact was examined, the results were the same. This indicates that the cell velocity recovers from the contact slowdown in a fairly short (<15 min) time.

Table 4. Comparison of contact and non-contact velocities

<table>
<thead>
<tr>
<th>Segment</th>
<th>No. pairs</th>
<th>Mean velocity</th>
<th>Paired t</th>
<th>d.f.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-contact</td>
<td>828</td>
<td>(0.77 \pm 0.18)</td>
<td>11.735</td>
<td>827</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Contact</td>
<td></td>
<td>(0.92 \pm 0.16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-contact</td>
<td>828</td>
<td>(0.81 \pm 0.20)</td>
<td>10.40</td>
<td>827</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Contact</td>
<td></td>
<td>(0.92 \pm 0.16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-contact</td>
<td>828</td>
<td>(0.81 \pm 0.20)</td>
<td>0.154</td>
<td>827</td>
<td>0.88</td>
</tr>
<tr>
<td>Post-contact</td>
<td></td>
<td>(0.77 \pm 0.18)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Although the decrease in velocity was significant, it was not large (contact velocity was 81% of the non-contact velocity). These results are similar to those of Ramsey & Harris (1973) for leukocytes, with the exception that they did not find a significant difference between velocity during contact and velocity after contact.

In his review of the behaviour of cellular slime moulds, Shaffer (1962) suggests at several points that cells tend to avoid and quickly break contacts. He presumes that some form of contact inhibition related to that observed in tissue culture (Abercrombie & Heaysman, 1953) may be present. Our results on contact frequency and duration indicate to the contrary that cells at lower densities have an affinity for each other and tend to stay in contact as though adhering physically. This together with the facts that cells do not stop during contact and do move normally following contact, all show that slime mould cells do not exhibit contact inhibition.

The affinity effect, though significant, is fairly mild. If instead of a cell diameter of 10 \(\mu\)m, a value of 20–30 \(\mu\)m (depending on the film) is used in equation (4), the calculated contact frequencies become equal to those measured. This 20–30 \(\mu\)m distance gives a rough indication of the range over which cells may interact during this stage of their development. This is less than the 57-\(\mu\)m range measured for cells during aggregation (Alcantara & Monk, 1974).

Correlations

Mean values for the motion parameters from the various models were calculated separately for each film. When the mean values for one parameter are plotted vs. mean values for another, film-by-film, the resulting scatter plot gives a qualitative indication of the relationship between these quantities. A linear correlation coefficient can be obtained for each pairwise combination as a quantitative measure of the relationship between them. The results of this cross-correlation study are summarized in Table 5.
Table 5. Cross-correlations among motion parameters

<table>
<thead>
<tr>
<th></th>
<th>$N$</th>
<th>$N^*V$</th>
<th>$V$</th>
<th>$cf$</th>
<th>$cd$</th>
<th>$cf cd$</th>
<th>$D^*$</th>
<th>$t^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N^*V$</td>
<td>0.979</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(&lt; 0.001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V$</td>
<td>-0.362</td>
<td>-0.246</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.20)</td>
<td>(0.35)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$cf$</td>
<td>0.466</td>
<td>0.610</td>
<td>0.050</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.15)</td>
<td>(0.05)</td>
<td>(&gt; 0.50)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$cd$</td>
<td>0.223</td>
<td>0.061</td>
<td>-0.596</td>
<td>-0.575</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.50)</td>
<td>(&gt; 0.50)</td>
<td>(0.06)</td>
<td>(0.07)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$cf cd$</td>
<td>0.890</td>
<td>0.921</td>
<td>-0.306</td>
<td>0.663</td>
<td>0.119</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
<td>(0.35)</td>
<td>(0.03)</td>
<td>(&gt; 0.50)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$D^*$</td>
<td>-0.392</td>
<td>-0.318</td>
<td>0.916</td>
<td>-0.105</td>
<td>-0.384</td>
<td>-0.258</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.15)</td>
<td>(0.22)</td>
<td>(&lt; 0.001)</td>
<td>(0.50)</td>
<td>(0.22)</td>
<td>(0.45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t^*$</td>
<td>-0.028</td>
<td>-0.183</td>
<td>-0.608</td>
<td>-0.686</td>
<td>0.761</td>
<td>-0.163</td>
<td>-0.351</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.50)</td>
<td>(0.02)</td>
<td>(0.02)</td>
<td>(0.006)</td>
<td>(&gt; 0.50)</td>
<td>(0.20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S$</td>
<td>-0.315</td>
<td>-0.195</td>
<td>0.970</td>
<td>0.183</td>
<td>-0.072</td>
<td>-0.226</td>
<td>0.839</td>
<td>-0.729</td>
</tr>
<tr>
<td></td>
<td>(0.25)</td>
<td>(&gt; 0.50)</td>
<td>(&lt; 0.001)</td>
<td>(&gt; 0.50)</td>
<td>(0.03)</td>
<td>(0.50)</td>
<td>(&lt; 0.001)</td>
<td>(0.003)</td>
</tr>
</tbody>
</table>

$N$: density

$N^*V$: density times mean velocity

$V$: mean velocity

$cf$: contact frequency

$cd$: contact duration

$cf cd$: contact frequency times contact duration (% time in contact)

$D^*$: diffusion constant

$t^*$: persistence time

$S$: $\sqrt{2D^*/t^*}$
Cell motion in Dictyostelium

The numbers at each intersection of the table are the linear correlation coefficient between the pair, and below that, in parentheses, the two-tailed probability that such a correlation coefficient could arise from a random population. The number of films used in obtaining these numbers was 14 except for the correlations involving contact measurements (cf, cd, cf*cd) where only 11 films, in good focus throughout were analysed. The probability figure is based on the number of films used in the calculation.

Both some expected and unexpected outcomes are noticeable in the table. The correlation of S and \( V_{\text{rma}} \) cell-by-cell was shown in Fig. 5, and Table 5 shows that S and \( V \) are also highly correlated film-by-film. Diffusion constant, \( D^* \), correlated significantly with mean velocity but with no other quantity. Persistence time, \( t^* \), correlated inversely with velocity, as was shown cell-by-cell in Fig. 6. Persistence time also correlated inversely with contact frequency, implying cells that frequently changed direction made more contacts, and correlated directly with contact duration, implying that cells which moved generally in straight lines tended to make longer contacts. Neither \( D^* \), \( t^* \), nor S showed significant correlation with cell density.

Contact frequency correlated with density times velocity, in agreement with the model based on the kinetic theory of gases, but not with velocity by itself. Thus, the similarity of the time courses of velocity and contact frequency (as noted in Contacts) appears to be the result of processes more subtle than a direct relationship between the 2 quantities.

Contact duration correlated inversely with velocity and directly with \( t^* \) implying that contact durations are shortened both by high velocity and short persistence. As would be expected, contact duration and contact frequency were inversely correlated.

For all 14 films velocity and density have a negative correlation coefficient \( (r = -0.362) \), which is marginally not significant \( (P = 0.20) \). The correlation between velocity and inverse density for all 14 films is slightly more positive \( (r = 0.390) \), but still not significant \( (P = 0.17) \). If 13 of the 14 films are used, however, the correlation coefficient between velocity and inverse density increases dramatically \( (r = 0.536) \) and the correlation becomes significant \( (P = 0.07) \). The film not used in this correlation is one of the very low-density films. In 4 other low-density films the cells have a high mean velocity \( (> 10 \mu\text{m}/\text{min}) \), but this film has a mean velocity of \( 6.5 \mu\text{m}/\text{min} \). Thus, most films appear to show an inverse relation between velocity and density, as suggested by Shaffer (1962). This mild effect may be acting through cell contacts, since in high-density films cells spend more time in contact, and cells in contact move more slowly.

Comparison with other strains

Our purpose has been to analyse a large number of *D. discoideum* NC-4 cells under a standard set of conditions. The data are sufficient to represent average NC-4 behaviour as well as the range of behaviours under our conditions. A pattern of interrelated qualitative properties emerges as characteristic of *D. discoideum* NC-4 cell motion. For a different cell strain, one can expect to find one or more salient and distinctive properties by comparison to the range of NC-4 behaviours after analysing...
Fig. 11. Six-hour cell tracks of various strains: A, *D. discoideum* NC-4; B, *D. discoideum* mutant N15F; C, *D. discoideum* Ax-3; D, *D. minutum*; E, *D. discoideum* mutant P2. Notice that A and B are twice the linear scale of C and D, and E is 10 times the scale of C and D or 5 times A and B.
Cell motion in Dictyostelium

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a number of cells smaller than that needed for a full characterization. This approach has proved useful as a screening technique following mutagenesis as well as an identification technique for distinguishing mutants from wild-type in mixing experiments. The behaviours of various mutants hopefully can be related to information obtained biochemically. Overall, the results of this section suggest that motion is a distinctive characteristic of cell type.

Table 6. Summary of motion parameters for various strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>D.d. NC-4</th>
<th>P2</th>
<th>N15F</th>
<th>D.d. Ax-3</th>
<th>D. minutum</th>
</tr>
</thead>
<tbody>
<tr>
<td>num cells</td>
<td>495</td>
<td>67</td>
<td>10</td>
<td>54</td>
<td>40</td>
</tr>
<tr>
<td>$V$ ($\mu$m/min)</td>
<td>7.19</td>
<td>15.3</td>
<td>8.66</td>
<td>1.47</td>
<td>6.22</td>
</tr>
<tr>
<td>$\sigma_r$ (15 min)</td>
<td>4.41</td>
<td>4.63</td>
<td>2.30</td>
<td>0.93</td>
<td>3.38</td>
</tr>
<tr>
<td>$v_{\text{max}}$ (15 min)</td>
<td>0</td>
<td>8.0</td>
<td>3.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$D^*$ ($\mu$m$^2$/min)</td>
<td>31.3</td>
<td>24.1</td>
<td>15.3</td>
<td>6.5</td>
<td>17.2</td>
</tr>
<tr>
<td>$r_s/r_h$</td>
<td>4.89</td>
<td>13.7</td>
<td>2.3</td>
<td>7.1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>$r_s/r_h$</td>
<td>1.15</td>
<td>0.874</td>
<td>1.15</td>
<td>1.05</td>
<td>1.075</td>
</tr>
<tr>
<td>$P_s$</td>
<td>0.951</td>
<td>&lt; 0.001</td>
<td>0.006</td>
<td>0.401</td>
<td>0.295</td>
</tr>
</tbody>
</table>

This section describes several examples of this approach in studying 2 mutants of D. discoideum NC-4, D. discoideum strain Ax-3, and D. minutum. Fig. 11 shows representative or distinctive tracks of cell motion for NC-4 and the 4 other strains. Table 6 provides a summary of the principal motion parameters for the various strains.

D. discoideum mutant P2. P2 is a mutant of D. discoideum NC-4. A total of 67 cells in two films were digitized. P2's most interesting characteristic is its motion. P2 has a very high mean velocity of 15.5 $\mu$m/min, about twice that of NC-4. Particularly distinctive is that no slow or stopped cells were observed, the slowest 15-min mean velocity measured being 8.0 $\mu$m/min. Secondly, P2 has a very long persistence time of 13.7 min. Computing this value by the method in the Appendix indicates that the distribution of persistence times for P2 is less exponential than for any of the other strains studied. The long persistence time together with the high velocity leads to very long cell tracks (Fig. 11E; N.B. the change of scale) and an extremely high diffusion constant of 975.6 $\mu$m$^2$/min. The combination of high velocity and long persistence is completely contrary to the persistence vs. velocity relationship established for NC-4 earlier, p. 292. Fig. 6 is reproduced as Fig. 12 with the values for P2 cells (19 cells with at least 45-min cell tracks) appearing largely in the region completely free of NC-4 cells.

P2 does not aggregate and shows no response to cAMP. Instead P2 forms single cell microcysts (Frantz, 1978), similar to what is sometimes observed for D. mucoroides and other species (Bonner, 1967, p. 40). P2 cells also have substantial mutual contact and adhesion, with broad bands of group motion frequently observed. The nearest-neighbour test reveals that in various frames significant clumping takes place in P2 fields ($P < 0.001$) at densities of $\sim 10^4$ cells/cm$^2$, the only strain studied for which this was the case.
D. discoideum mutant N15F. N15F is another mutant of D. discoideum NC-4. N15F's salient behaviour is seen in its overall cell track Fig. 11B, a jittery side-to-side motion superimposed on an overall motion. The jitter results in a low persistence time value of 1.8 min. Nonetheless its overall mean velocity is not significantly different from NC-4 (t = 1.05, d.f. = 9, P = 0.32 two-tailed). As for the overall motion, N15F wanders about somewhat but only within a few hundred micron range and so the diffusion constant is a very low 38.1 μm²/min. Indeed the 10 cells digitized each remained within the 1-mm field of view for the entire length of one 2400-frame (10-h) film. N15F also makes virtually no or only very short contacts. The cells have a spatial distribution that is more uniform (P = 0.006) than expected at a density of 1.3 × 10⁴ cells/cm² by the nearest neighbour test. Additionally cells only occasionally come to a stop or exhibit rapid motion; across the field the velocities appear to be less variable than for NC-4. N15F eventually does aggregate.

D. discoideum Ax-3. The axenic strain Ax-3 is commonly used in much of the biochemical work on D. discoideum, and thus it is of interest whether Ax-3 is similar to NC-4 in its behaviour. Fifty-four Ax-3 cells were digitized in 2 films. Cells of Ax-3 are very slow with a mean velocity of only 1.47 μm/min, a fifth that of NC-4. Cells
frequently come to rest and sustained fast cell motions were not observed. Overall motion, other than being limited by the low velocity, is a normal wandering motion without jitter and with perhaps somewhat longer straight sections. Persistence time is about half again longer than NC-4 in min, although this should be taken in the light of its low velocity. Fig. 11c shows a distinctive behaviour observed in a number of cells: motion in loops that are repeated several times. Contacts in Ax-3 appear to be qualitatively similar to those for NC-4, with frequency and duration again relative to the low velocity. Spatial distributions are random by the nearest-neighbour test for densities $\sim 5 \times 10^3$ cells/cm$^2$.

$D.\ minutum$. A different Dictyostelium species $D.\ minutum$ strain DC-4 was also studied by digitizing 40 cells from one film. Mean velocity for $D.\ minutum$ was somewhat less than for $D.\ discoideum$ NC-4 but not significantly ($t = -1.39$, d.f. = 39, $P = 0.17$ two-tailed). $D.\ minutum$ shows a considerable amount of retracing of paths just taken and back-and-forth area filling motion, Fig. 11d. It shows almost no long straight stretches like NC-4. Its persistence time is so small as to be almost unmeasurable at its velocity. The characteristic area filling motion is very tightly wound so that $D.\ minutum$ makes little ultimate progress, having a diffusion constant of only $20-1 \mu m^2$/min. Contacts are similar to those for NC-4 except that, perhaps due to the back-and-forth motion, 2 neighbouring cells may repeatedly make contact. Cell spacing is random by the nearest-neighbour test at a density of $\sim 8 \times 10^3$ cells/cm$^2$.

CONCLUSION

This paper presents results concerning the motion of Dictyostelium discoideum cells during the period before aggregation. This morphological study provides information about the behaviour of individual cells before they enter their overtly social phase. We are using these results as a basis for comparison with our current research concerning the motion of $D.\ discoideum$ cells during aggregation, as well as with the motion of mutants of $D.\ discoideum$. Finally, this work applies several useful models for the description and evaluation of salient aspects of cell motion.

This work has been supported by NIH grant HD-04722 and NSF grant MCS 75-22362 to the University of Chicago, and by a predoctoral training grant GM-07183 to S.A.M. We would like to thank C. Frantz for her assistance in several aspects of this study, and H. Cornejo, P. Devreotes, R. Clark, and A. Robertson for their comments and criticism.
APPENDIX. COMPUTATION OF DIFFUSION CONSTANT AND PERSISTENCE TIME USING VELOCITY AUTOCOVARIANCE

An alternative method for computing the parameters $D^*$ and $t^*$ involves the use of velocity autocovariance. For each cell, velocity vectors $v_i$ giving the speed and direction of motion in 2 dimensions can be computed for successive uniform time intervals (in our case 30–120 s depending upon mean cell velocity, so that on the average the cell moves 1 cell diameter during each interval). The velocity autocovariance function is then computed as

$$A(s) = \sum_{t=1}^n v_i \cdot v_{i+s} / n,$$

where vectors are multiplied using dot product. A typical graph for actual data is shown in Fig. 13. $A(s)$ is essentially zero for large enough time shifts, $s$, since cell behaviours separated by these amounts are uncorrelated. $A(s)$ is increasingly positive for smaller shifts because of persistence. By definition, $A(0)$ has the value $\overline{v^2}$.

![Fig. 13. Typical graph of the velocity autocovariance function $A(s)$ for one cell vs. time lags in increments of 90 s, showing the effect of persistence.](image)

The method of Gail & Boone (1970) involves computing the graph of mean squared displacement $\overline{R^2(t)}$ to determine $D^*$ and $t^*$. A transformation from the velocity autocovariance function $A(s)$ into the mean squared displacement function $\overline{R^2(t)}$ can be derived (Egelstaff, 1967), which is given by

$$\overline{R^2(t)} = 2t \int_0^t (t-s/t) A(s) \, ds.$$  \hspace{1cm} (A 2)

This transformation is valid regardless of the underlying distribution of instantaneous persistence values. For actual cell motion data one can compute the velocity autocovariance function $A(s)$ using equation (A 1), substitute $A(s)$ into equation (A 2), and
integrate equation (A 2) numerically to produce a graph of mean squared displacement \( R(t) \). From this graph, values of \( D^* \) and \( \tau^* \) may be computed using the slope and intercept method of Gail & Boone (1970) as in Fig. 4 (in section on Diffusion constant and persistence time).

Moreover, one can derive a formula for \( D^* \) directly from equation (A 2) by taking the derivative of \( R(t) \) with respect to \( t \) as \( t \to \infty \) and equating the result to \( 4D^* \) (Gray, 1968) to obtain the equation

\[
D^* = \int_0^\infty A(s) \, ds / 2. \tag{A 3}
\]

With this formula, \( D^* \) can be computed directly from an actual velocity autocovariance function \( A(s) \), using numerical integration to evaluate equation (A 3). Notice again that this method for computing \( D^* \) does not depend on assuming the underlying persistence distribution. When values of \( D^* \) were calculated for each of our cells using this method, they differed insignificantly from the \( D^* \) values obtained using the Gail & Boone (1970) method (\( t = 1.37, P = 0.17 \) by a two-tailed \( t \)-test paired cell-by-cell).

More can be said if the assumption is made that instantaneous persistence values are exponentially distributed. Making this assumption allows Gail & Boone (1970) to derive the following equation for the graph of mean squared displacement.

\[
\bar{R}(t) = 4D^* (t - \tau^*(1 - \exp(-t/\tau^*)\)). \tag{A 4}
\]

Lovely & Dahlquist (1975) also make the assumption of an exponential distribution and derive a result that shows the velocity autocovariance function defined by equation (A 1) will be

\[
A(s) = \sqrt{\tau} \exp(-s/\tau), \tag{A 5}
\]

where \( \tau \) is the mean of the exponential distribution. Although Lovely & Dahlquist (1975) do not connect this result to that of Gail & Boone (1970), it can be shown that these results do correspond. In particular substituting equation (A 5) into equation (A 2) and integrating analytically results in the formula

\[
\bar{R}(t) = 2\sqrt{\tau} \tau (t - \tau (1 - \exp(-t/\tau))). \tag{A 6}
\]

Thus, equation (A 6) is just the same as equation (A 4) with

\[
\tau^* = \tau \tag{A 7}
\]

and

\[
D^* = \sqrt{2}\tau^*/2. \tag{A 8}
\]

Because of equation (A 7), \( \tau^* \) can be computed by obtaining the best fit by chi-square minimization of equation (A 5) to an actual graph of the velocity autocovariance function \( A(s) \) computed using equation (A 1). When values of \( \tau^* \) were calculated for each of our cells using this method, they differed insignificantly from the \( \tau^* \) values
obtained using the Gail & Boone (1970) method ($t = 1.60, P = 0.11$, by a two-tailed $t$-test paired cell-by-cell).

Equation (A 8) is a relationship that will hold among the parameters $D^*, t^*$, and $\bar{V}_s (= \nu^*_r)$ if the assumption of an exponential distribution for persistence is valid.

**LIST OF SYMBOLS USED IN TEXT**

- $A(s)$: velocity autocovariance function
- $cd$: contact duration
- $cf$: contact frequency
- $D$: diffusion constant
- $D^*$: modified diffusion constant
- $d$: cell diameter
- $N$: cell density
- $n$: number of discrete steps in Drunkard's walk model
- $P_s$: probability that a spatial distribution is indistinguishable from random
- $P$: probability that the observed deviation or correlation could arise in a random sample
- $Q$: ratio $r_A/r_E$
- $\bar{R}$: expected displacement from a starting point
- $r$: linear correlation coefficient
- $r_A$: actual value of distance between cells
- $r_E$: expected value of distance between cells
- $S$: $\sqrt{(2D^*/t^*)}$
- $s$: length of a step in Drunkard's walk model
- $t$: time; 'Student's' $t$-statistic
- $t_e$: mean of the exponential distribution of instantaneous persistence values
- $t^*$: persistence time
- $\nu^*$: mean velocity (speed)
- $\nu_{\text{max}}$: maximum velocity observed in an interval
- $\nu_{\text{median}}$: median of the velocity distribution
- $\nu_{\text{min}}$: minimum velocity observed in an interval
- $\nu_{\text{mode}}$: mode of the velocity distribution
- $\nu_{\text{rms}}$: root mean square of the velocity distribution
- $Z$: normal deviate statistic
- $\sigma_v$: standard deviation of velocity

**REFERENCES**


Cell motion in Dictyostelium


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