SIMILARITY OF SISTER-CELL TRAJECTORIES IN FIBROBLAST CLONES

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SUMMARY

The observation that sister cells of a fibroblast division execute somewhat symmetric trajectories has led to the controversial hypothesis that cell pathways on a substrate are preprogrammed in detail. We have investigated symmetry and structure in the paths of 170 human AG1519 sister-fibroblast pairs recorded on film and analysed by interactive computer techniques. In a set of plots of sister-cell trajectories recorded immediately after cell division, we found an average of 31 % mirror symmetry and 14 % rotation identity after rejecting tracks that crossed over each other, a result in substantial agreement with published data. Such 'symmetries' are quite inexact, however. A symmetry-controlled perceptual experiment using a stimulus set based on real tracks showed a bias towards mirror symmetry over identity. A large fraction of paired random trajectories of few independent steps and turning angles can be expected to exhibit true turn symmetry on the basis of chance alone. But as the number of turning angles increases the number of rigorously symmetrical pairs decreases sharply. Subjects classified nearly 50% of 10-step computer generated random persistent trajectory pairs as symmetrical while only a fraction of a percent are rigorously so. In fact, they were classified as though they contained only three to four random independent steps. The bias of the human observer and chance may thus account for the perception of a high occurrence of sister trajectory symmetry in vitro.

Since branching patterns of sister trajectories do not possess rigorous symmetry, we feel they cannot be taken as evidence of preprogramming. However, the dissimilarity of the structure of sister-cell walks does not rule out the possibility that sister cells are alike in other respects. We found a significant correlation between the time-average velocities of newly born sister cells, and suggest that it and other similarities may be due to the equipartitive character of mitosis. The correlation decays over a 2-5-day period after mitosis. But phenotypic sister-cell similarities do not appear to determine the sequence of turning angles in cell walks. If they did a claim might be made for the preprogramming of locomotion.

INTRODUCTION

The 'programming' model of sister-cell locomotion immediately following cell division presupposes that the long-term detailed trajectory of an individual cell is cytostructurally coded (Albrecht-Buehler, 1977a,b,c, 1978). This idea has serious theoretical implications for the chemostructural basis of locomotion and for the possibility of non-nuclear information storage in living eukaryotic systems. It implies a higher degree of cytostructural organization than has heretofore been observed.

It is well known that individual cells and neuronal processes are capable of executing

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complex directional locomotory patterns during embryonic development, regeneration and wound healing. Hence evidence for programmed migration of tissue cells independently of external guidance would be of extreme importance for understanding these basic processes.

In this paper we specifically raise the issue of whether symmetry of sister-cell pathways, as perceived by the scientific observer, can be taken as evidence for preprogramming. No formulation of the programming model known to us has required exact geometric similarity in the paths of sister pairs, nor has the degree of similarity required before trajectories can be counted as 'mirror' or 'identical' been explicitly defined. Trajectories are scored as symmetries on the basis of perceptual judgment of similarity (Albrecht-Buehler, 1977a). In the present study we wish to determine whether chance and the human observer can provide the basis for the results observed.

In the more classical view (e.g., see Gail & Boone, 1970, 1971a,b; Hall, 1977), crawling tissue cells of various types are imagined to be free-ranging entities, which move in a random or unpredictable direction. Their movements can be directed or influenced by chemotaxis (Dixon & McCutcheon, 1936; Nossal & Zigmond, 1976; Hall & Peterson, 1979; Shiffman & Gallin, 1979), chemokinesis (Allan & Wilkinson, 1978), substrate guidance (Weiss, 1961; Weiss & Garber, 1952; Weiss & Taylor, 1956; Albrecht-Buehler, 1979), substrate specificity (Gail & Boone, 1972) or contact with other cells (Weiss, 1958; Middleton, 1973; Steinberg, 1973). Patlak (1953a,b), Gail & Boone (1970) and Nossal (1976) have dealt with the notion of a random persistent walk, where persistence describes the tendency of a cell to continue to move in a given general direction over short periods of time.

In addition to chemotaxis, contact guidance and other external directive influences, contact inhibition also affects cell translocation. Contact inhibition has been used to explain the observed cessation of growth and motion of monolayered fibroblasts approaching confluence (Abercrombie & Heaysman, 1954; Weiss, 1958; Gail & Boone, 1971a; Armstrong & Armstrong, 1973; Middlelon, 1973). The current understanding is that confluent cells continue to mill about, but their gross translocation is restricted by an avoidance of underlapping, or by the constraints of contact-attachments between cells (Steinberg, 1973; Martz & Steinberg, 1974; P. B. Bell, 1977). Differences in the adhesiveness of various substrates that affect fibroblast motility have been examined by Weiss (1961), Gail & Boone (1972) and Harris (1973). The possible influence on motility of attachment proteins such as fibronectin (Yamada & Olden, 1978; Yamada, Olden & Hahn, 1980) and laminin (Terranova, Rohrbach & Martin, 1980), which cells themselves generate, still requires elucidation.

Previously, we (E. Bell et al. 1978; 1979a,b) and others (Absher, Absher & Barnes, 1974, 1975; Mertz & Ross, 1973; Smith & Hayflick, 1974) have shown that considerable heterogeneity of interdivision time, cycling state, size and age occurs in clonal fibroblast populations and even between members of sister-cell pairs. In the present paper we extend these studies and examine the heterogeneity of sister-cell velocities.
MATERIALS AND METHODS

Data-acquisition system for motility and lineage studies

A data-gathering system (E. Beller et al. 1979a; Levinstone, 1981) designed for cell motility studies consists of a general purpose digital computer, a table with a hard surface rear-projection screen, an electronically controlled motion-picture projector with computer interface, an \((x,y)\) coordinate digitizing instrument (Numonics Corp.), and computer programs for interactive collection and analysis of cell trajectory data from time-lapse films. A cell-tracking program records one coordinate pair for each frame in which a given cell can be discriminated. It provides for storage, retrieval and display of the trajectory data. Cell genealogy is also recorded in the data base.

The path of each cell is traced by following the position of the nucleus with an overlying cursor attached to the arm of the digitizer. One point is plotted for the position of the daughter in each frame, and straight lines are drawn between the points (Fig. 1). The trajectories were also plotted as thick tracks (Fig. 1) having the width of an average cell, to give a form analogous to the static 'phagokinetic' tracks in published data (Albrecht-Buehler, 1977a). A black square centered on each recorded point creates a trajectory with a width of 21 tablet points (= 34 \(\mu\)m).

The ability of an observer to match up sister pairs of trajectories that have been separated to test symmetry would in part be determined by features that have nothing to do with trajectory symmetry, such as trajectory width and overall length. Similarity of path-width due to similarity of size of newly born sisters quickly directs the observer to the mate of the sample under scrutiny. Also, match-up of sisters might be facilitated by similarity of overall trajectory length due to comparable activity levels. It is not easy to see how these cues can be eliminated in comparing actual cell trajectories. We have therefore not used the match-and-pair approach in conducting tests calling for symmetry evaluation, and the widths of all cell paths have been made similar.

Calculation of cell velocity

A program was written to calculate the time-averaged velocity, that is the distance traversed in a given period divided by the time. The trajectories were processed so that points closer than 4-6 \(\mu\)m were eliminated, and the total path-length was computed by summing the distances between remaining points. The foregoing procedure ('windowing') eliminates motions smaller than the hypotenuse of a 2 \(\times\) 2 tablet-unit triangle, hence much smaller than a cell, thereby compensating for the effects of slight jitter of the tracer's hand and mechanical sluggishness of the tablet arm; it primarily diminishes the effects of grid quantization on the computation of distance.

Filming the growth of a clone

We established our own data base of cell coordinate versus time files from films of fibroblasts in culture. The cells were human infant foreskin fibroblasts (strain AG1519) furnished by the Human Genetic Mutant Cell Repository, Institute for Medical Research, of Camden, NJ. Filming of clones corresponding to the 10th, 20th and 30th population-doubling levels (PDL) was done at 5 min intervals.

Inverted or upright microscopes were maintained at 37°C in an atmosphere containing 5% CO\(_2\) in air. A Falcon Cooper dish with nine coverglasses, which had received platings of a dilute suspension of cells, was sealed with silicone grease and positioned on the stage of a microscope. A founder cell of a clone that had begun to divide was picked for filming. After filming, the colony was circled and the coverglass was usually removed for immune staining or other post-facto operation.

The microscope is equipped with a condenser and objective fitted with Hoffman Modulation Contrast Optics (Hoffman & Gross, 1975). This system was chosen over phase-contrast, because it provides a well-resolved halo-free image at low magnification. The working field was about 2 mm \(\times\) 2.5 mm. An Arriflex 16 mm movie camera was mounted independently above the eyepiece of the microscope. A simple clock-motor and timing-loop trigger the light source and the shutter of the camera once every 5 min. Cells were filmed over periods of several days to 2 weeks (a total of about 4500 frames), with occasional manual refocusing. H & W VTE Panchromatic or Kodak SO115 negative film was used; positive work prints were made for interactive computer analysis. A micrometer scale recorded on the last frames of each film creates a record of system magnification.
Pseudo-random generation of trajectories with equal step length

A computer program was written to generate paired trajectories as a series of short straight-line 'steps' of equal length, separated by turning angles chosen according to a discrete distribution function of arbitrary complexity, the result being one form of a persistent walk (Barber & Ninham, 1970). A sequence of angles was derived from a pseudo-random number generator according to Knuth's (1971) algorithm M. The first step of a daughter cell was arbitrarily placed at a 90° or 180° angle to the first step of its sister. Then, independently, an upper and a lower trajectory were 'grown' from the ends of these by placing a sequence of nine continuous steps separated by pseudo-random angles, including 0°, following the probability distributions designated in Results. Each model daughter of a pair was thus provided with a uniquely determined trajectory.

RESULTS

Visual examination and classification of trajectories of sister-cell pairs of 11 colonies

One hundred and seventy thick-track sister trajectory pairs were classified by two observers as instances of mirror symmetry (M), rotation identity symmetry (I), asymmetry (AS), insufficient data (IN), or not acceptable (NA). AS covers the cases in which sister tracks were sufficiently long, but were not of comparable form; the IN class covers that category of cells that did not move substantially, and NA covers those whose trajectories were difficult to follow because of track crossovers. Reference was made to the thin (point) plots when a separation point or path direction was ambiguous in the thick track print-out.

Results are given in Table 1, and typical pairs of sister-cell trajectories are seen in Figs 1 and 2.

Of the 170 track pairs, Observer I found 35 (21%) to be mirror symmetric to at least one significant turn, and 18 (11%) to exhibit identity symmetry, for a total of 31% symmetries, while Observer II found 30 (18%) M and 12 (7%) I. Their M/I ratios, 1.9/1 and 2.5/1, respectively, agree with Albrecht-Buehler's (1977a) finding of about twice as many M as I pairs.

To compare our incidence of symmetry with that of Albrecht-Buehler (1977a), we subtracted the NA pairs from the total, since such images were excluded from his count of 40% M, 14% I. This normalization leads to 27% M, 14% I for Observer I, and 35% M, 14% I for Observer II, which are in reasonable agreement with each other and with published results (Albrecht-Buehler, 1977a).

Table 1. Assignment of 170 track pairs to five classes by two observers

<table>
<thead>
<tr>
<th>Observer</th>
<th>M</th>
<th>AS</th>
<th>IN</th>
<th>NA</th>
<th>I</th>
<th>M+I</th>
<th>M/I</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>21</td>
<td>25</td>
<td>20</td>
<td>24</td>
<td>11</td>
<td>31</td>
<td>1.9/1</td>
</tr>
<tr>
<td>II</td>
<td>18</td>
<td>15</td>
<td>12</td>
<td>49</td>
<td>7</td>
<td>25</td>
<td>2.5/1</td>
</tr>
</tbody>
</table>
Fig. 1. Thin and thick track representations of cell locomotion.

The point of origin of the sisters is indicated by an arrow. A appears less asymmetric in its original, thin representation than in the thick track representation, which has rough mirror symmetry; B has three major elements that contribute to mirror symmetry; C may be considered to be mirror symmetric if the wiggle on the upper track is ignored, identity symmetric if not. D can also be ambiguously interpreted as having either symmetry.

Analysis of human symmetry classification, using real and 'symmetry-transformed' trajectory data

Since our subjects and others (Albrecht-Buehler, 1977a) score relatively imperfect sister-pair trajectories as symmetries, we investigated the performance characteristics of the human observer.

A subset of 25 daughter-pair trajectories containing mirror images, identities, and asymmetries was picked. A 'thick-track' plot of each was made with the mother cell's path removed but the point of origin noted. For each sister pair, a symmetry-transformed image was created by reflecting one sister track about the horizontal or
vertical axis centered on the origin of the trajectory, while that of the other sister remained fixed (Fig. 2). Thus, if the original pair of tracks were a mirror pair, then the transformed image would contain an identity pair; identities would become mirrors and asymmetries would remain asymmetries.

There were thus 25 original and 25 transformed trajectory pairs. These were cut into 4 inch × 4 inch squares, randomized by shuffling and rotation, and then numbered. An arrowhead was placed on each image near the known division point of the mother cell. Examples of the 25 sets of original and transformed images are included in Fig. 2. Twenty subjects classified their responses according to the following categories: (a) good mirror, (b) mirror, (c) asymmetry, (d) identity, and (e) good identity. The data were reduced to three classes (a) + (b), (c), and (d) + (e), for further analysis, and are presented in Table 2.

The ratio of the pooled subject responses for \( M/I = ((a) + (b))/(d) + (e) = 1.22 \). The difference between the two classes, \( M - I = 382 - 312 = 70 \), corresponds to an average per subject of 7% more ‘Mirrors’ than ‘Identities’. The average total percentage of combined M or I symmetry is 69.4%. The 7% difference is statistically significant at the 0.02 level using student’s \( t \)-test for correlated pairs \( (t = 2.76 > t_{0.02,19}) \). The data suggest that subjects prefer classifying trajectory pairs as mirror images rather than as rotation identities, whereas, by construction, there should be equal numbers of each.

**Table 2. Classification by 20 subjects of symmetry for a set of 25 original and 25 transformed trajectories**

<table>
<thead>
<tr>
<th>Class</th>
<th>Trajectory type</th>
<th>Number of responses</th>
<th>% Total responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) + (b)</td>
<td>Mirror</td>
<td>382</td>
<td>38.2</td>
</tr>
<tr>
<td>(c)</td>
<td>Neither</td>
<td>306</td>
<td>30.6</td>
</tr>
<tr>
<td>(d) + (e)</td>
<td>Identity</td>
<td>312</td>
<td>31.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>1000</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

Rigorous symmetries under simple random-walk assumptions

In an analytical model, the extent to which the sample set consists of trajectories with few turning angles will determine the degree of rigorous symmetries expected on

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**Fig. 2.** Thick track representation of pairs of 1-day sister-cell trajectories and ‘symmetry-transformed’ trajectories.

The point of origin of the sisters is indicated by an arrow. All images except C and 1 are original sister pairs. Image C was produced by mirroring the lower track of image J about the vertical axis, followed by 180° rotation of the entire configuration. Image 1 was produced by similarly transforming image G.

Images A, C, D, F, I and L were classified as M by a plurality of 20 subjects; H and J were classified as I by a plurality; and B, E and K were so classified as X. Image G was called M by eight, I by eight, and X by four subjects, and thus may be said to be highly ambiguous in terms of perceived ‘rough symmetry’.
the basis of chance alone. For example, if we assume that left or right turns are equally probable, symmetry as a function of the number of segments will occur as shown in Table 3, for paths of $N$ turning angles and $N + 1$ steps. For any instance of $N$ turns of the left track, there must be $N$ specific turns of the right track for symmetry (M or I) to hold. The net probability of this agreement is $0.5^{N-1}$.

This model predicts an equal number of mirror and identical track pairs for any number of segments. For example, all walks of two segments and one turn are shown in Fig. 3. From the last column of the Table, it is clear that if a set of walks with an

Table 3. Probability of identity, mirror or either symmetry in walks with $N$ comparable turns

<table>
<thead>
<tr>
<th>No. of turns</th>
<th>No. of segments in a track</th>
<th>No. of unique track pairs</th>
<th>$P(I)$</th>
<th>$P(M)$</th>
<th>$P($symmetry$)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>4</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>16</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>64</td>
<td>0.125</td>
<td>0.125</td>
<td>0.25</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>256</td>
<td>0.0625</td>
<td>0.0625</td>
<td>0.125</td>
</tr>
<tr>
<td>$N$</td>
<td>$N + 1$</td>
<td>$2^{2N}$</td>
<td>$0.5^N$</td>
<td>$0.5^N$</td>
<td>$0.5^{N-1}$</td>
</tr>
</tbody>
</table>

Fig. 3. The set of all trajectory pairs in which each sister makes precisely one turn, L or R, as indicated.

In this model, the precise length of path between noticeable turns is ignored, as are the precise values of the angles. In the figure, segment lengths are made roughly equal, and angles are all 90°. A and D are identities, B and C are mirror images.
assortment of numbers of comparable segments is classified on a basis of two to four segments, between 25% and 100% of the total will be symmetric. One of every eight walks of five segments will be symmetric in the absence of 'programming'. With the addition of each segment the probability of a path-pair being symmetrical is halved.

**Computer-generated random walks**

Since human subjects appear to be perceptually biased in assessing the symmetry or asymmetry of sister-cell trajectory pairs, we undertook to determine how chance combinations of model trajectories are perceived.

In generating random-walk trajectories one must decide which elements are statistical in nature, that is, governed by chance alone, and which are determined. In our simulation, the deterministic factors are the uniform step size, the stipulated sizes and probabilities of turning angles, and the assumption that these angles are independently distributed. Random elements are values of turning angles actually chosen from such a distribution and the resultant effect of the selection on the length of path segments between non-zero turning angles. The possible turning angles allowed for in our models fall within the range of turning angle preference found in published studies of fibroblast trajectories (Gail & Boone, 1970; Albrecht-Buehler, 1979).

Three sets of model trajectories were created by the pseudo-random walk computer program. The conditions for the first (set I) were as follows: the probability of not turning was made 50% and the probabilities of a +55° or a −55° turn were made 25% each. Twenty-four pairs of trajectories with an initial separation angle of 180° were generated. For the second set (II) the conditions were the same except that the initial separation angle was 90°. For the third set (III) the probabilities of five turning angles, −55°, −28°, 0°, +28° and +55°, were made equal and the separation angle between the first step of sisters was 90°. A transformed version of each trajectory pair was created by changing the signs of all turning angles in one of a pair of trajectories to their opposites. Examples of each set of model trajectories are given in Fig. 4. Even though the paths are specified randomly; that is, the sequences of turning angles of sister tracks are totally unrelated to each other, a large percentage of symmetries is perceived. Table 4 summarizes the scores of eight subjects who classified a total of 144 model trajectory pairs composed of 72 original and 72 transformed images.

As seen in sets II and III, the preferential selection of M over I is clear. If observers used consistent criteria for the selection of M or I the numbers should be equal.

**Table 4. How model trajectory pairs are perceived**

<table>
<thead>
<tr>
<th></th>
<th>Set I</th>
<th></th>
<th>Set II</th>
<th></th>
<th>Set III</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>X</td>
<td>I</td>
<td></td>
<td>M</td>
<td>X</td>
</tr>
<tr>
<td>% Selected</td>
<td>19</td>
<td>62</td>
<td>19</td>
<td></td>
<td>31</td>
<td>52</td>
</tr>
<tr>
<td>% Symmetry</td>
<td>38</td>
<td></td>
<td></td>
<td>48</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 4. Computer-generated pseudo-random track pairs of uniform step length. 
Originals (O) were created by laying down a series of ten steps for each sister, with the nine separation angles of each trajectory chosen independently via a random number generator. Symmetry-transformed versions (T) were created by holding the position of the dotted sister-track fixed and inverting the signs of the nine turning angles of the solid sister-track.

In set I, the initial separation angle is 180°; in sets II and III, it is 90°. In sets I and II, the probability of not turning (angle of 0°) was set to be 50%, and the probabilities of turning left or right (±55°) were set to 25% each. In set III, the turning angles were chosen from a quantized uniform distribution in which angles of ±55°, ±28°, 0°, +28° and +55° are equally probable.

Further analysis of the data showed that a large number (45) of symmetries were seen when asymmetries were transformed.

Three conclusions can be drawn: (1) a persistent random walk consisting of many elements; namely, nine turning angles and ten steps, can give rise to the appearance
of shape diversity and pair symmetry; (2) mirror symmetry is perceived preferentially
over identity; (3) the fraction of symmetries observed (38–48%) suggests that the
observers acted as if the tracks contained three to four steps or two to three turning
angles (see Table 3).

Comparison of time-average velocities of sisters as a function of time after division

Although the trajectories of sister cells may not be intrinsically symmetric, other
features of sister-cell motility may be similar. For example, we can determine with
reasonable objectivity whether sisters are equally active, since this measurement can
be made without involving human perceptual judgment in the way analysis of trajec-
tory shape did. We define activity as average cell velocity, and compute it for each cell
over the first 12 h of each day of its recorded existence. The period of a half-day was
chosen so that at least one mean velocity measurement would be available for cells that
divide in less than 24 h.

The time-average velocities of the sister cells of a third decade (30–40 population-
doubling levels) colony for 1–4 days following the division of the parent cell were used
to construct scatter plots and the coefficient of linear correlation was calculated
(Remington & Schork, 1970).

The coefficient of correlation \( r_{st} \) was 0·59 for the sister pairs, indicating a sig-
nificant non-zero correlation at the 0·01 level \( (N = 27; \text{two-tailed}) \) (Beyer, 1968) of
cell velocities on the first day after division. The correlation between paired sisters
that lived through the first half of a second day was also high \( (r = 0·72; N = 13; 0·01
level) \). The values on and after day 3 were not significantly different from zero.

Decay of similarity of velocity of sister-cell trajectories with time

In order to extend the sister-cell velocity correlation study to longer time periods
we pooled the data from 11 colonies, so that the number of remaining non-dividing
sister pairs in the field would be sufficiently high.

These correlation values are plotted versus cell age in days after division (Fig. 5).
The significance point at a given significance level is a function only of \( N \), the number
of points entering into the calculation of the correlation \( r_{st} \).

With the larger population sample, the correlation is non-zero for 3 days following
cell birth (significance is above the 0·05 level on the third day). After 3 days it
approaches zero.

Discussion

We have examined motile similarities between sister cells of human AG1519
fibroblasts (E. Bell et al. 1978) by means of time-lapse cinemicrography (E. Bell et
al. 1979a). Our results confirm in a general way the observation that, about 50% of
the time, the trajectories executed by sister pairs immediately after mitosis are per-
ceived as mirror symmetrical or identical (Albrecht-Buehler, 1977a), but we find no
support for the idea that locomotion of newly divided sister cells is programmed.
Rather, locomotion on a neutral substrate is seen by us as a random phenomenon. Sister-cell paths are classified as symmetrical by observers, even though they are geometrically asymmetrical, because the idealizing and simplifying tendencies of human judgment (Mach, 1897; Julesz, 1971, 1975; Zusne, 1970; Levinstone, 1981) lead to the perception of questionable symmetries as objects of more perfect form.

Given a small range of turning angles and small differences in the lengths of path intervals between turns it is possible, as we have done, to test the statistical and perceptual outcome of endowing cell models with artificially generated trajectories based on a random-walk model of motility. The results are surprising. When the number of statistically independent path components and alternatives used to compose a trajectory is small, the probability of observing rigorous symmetry is high. Model trajectories consisting of two segments separated by a left or right turning angle will yield perfect symmetry 100% of the time; with three segments and two turning angles, 50% of the time; with four segments and three turns, 25% of the time. Hence chance alone can account for a large number of rigorous symmetries when the number of turning angles is few. But the number of trajectories scored by an observer as symmetric can be large even when the components of trajectory are more numerous, because the observer tends to overlook small deviations from perfect symmetry.

The propensity of the observer to idealize form and to suppress or ignore small deviations from exact symmetry must be taken into account. Indeed, with model trajectories of considerably greater complexity consisting of ten steps separated by
Determinants of cell trajectory

nine pseudo-random angles as we report in this paper, we still observe a high degree
of similarity between many 'sister' pairs. The number that is observed as symmetrical
is near 50%.

While we do not dismiss the possibility that some initial cytostructural imprinting
on sister cells may be imparted by the symmetry of mitosis or by the tug and pull of
cell separation, we do not see either of these as causally related to the subsequent
events of cell or cell process translocation. Many observations suggest that cells and
cell processes are receptive to and depend on cues from the surroundings and readily
change their initial directions in response to them, particularly in the complex three-
dimensional environment of a developing organism (Ramon y Cajal, 1928; Van der
Loos, 1965).

The occurrence of some sister-pair configurations similar in all details, i.e.
rigorously symmetric, others that are more or less symmetric and are so scored
because of perceptual bias and, as observed, a class of trajectories that is asymmetric,
is consistent with expectations from a random-walk model of cell motility. We can
thus explain the variety of sister-cell trajectories observed by Albrecht-Buehler
(1977b) and by ourselves (Levinstone, 1981) without invoking preprogramming.
Although the branching structure of trajectories of cells, even sisters, may be
phenomena of chance described as random walks, some elements of motile behaviour
may be predictable, and/or sister-correlated.

We have seen that the average velocity of sister cells is highly correlated for several
days. Eventually the correlation decays, and the velocities of sisters become entirely
unrelated, reflecting the diversity representative of the population as a whole. We
attribute the initial similarity of cell velocity to the equipartition of maternal
cytoplasm and consequent highly probable equality of cell size, energy reserves and
other molecular pools. But there is no obvious causal relation between velocity, size,
available energy or any other feature of equipartition and the sequential determination
of turning angles.

While velocity of sister cells is significantly correlated during the first 2–3 days after
division, it does not determine the branching patterns of a trajectory. However, it is
interesting in its own right as a measurable feature that shows some persistence of cell
similarity in a heterogeneous population.

We have examined the correlation between velocity and segment length, and be-
tween velocity and turning angle for single cells and for sister pairs, and we find
absolutely no correlations. Hence velocity does not appear to determine path structure
(Jeon, Levinstone & Bell, unpublished results).

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