THE MATHEMATICS OF MOSAIC ANALYSIS
I: THE RELATIONSHIP BETWEEN STURTS AND DISTANCE

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ABSTRACT

In mosaic fate mapping the fraction of mosaics in which two structures are of different genotype is calculated. This frequency of separation has been called a “distance” and the units of this distance are called “sturts”. The fundamental assumption of fate mapping is that the frequency of separation increases continuously with the actual distance between the anlage for these structures on the blastoderm. This paper shows that the frequency of separation does not increase beyond a certain value. — For the current theory to work as proposed, each mosaic animal must be half mutant and half normal. This is rarely the case in collections of mosaics. It has been thought that if some flies are less than half mutant and others more than half, these two types would introduce compensating errors in mapping distance. We show that this is not true and describe the nature of the errors introduced. It is probable that these errors are the main reason that mapping distances reported from different sets of mosaics have not been reproducible. This paper presents methods for the proper handling of data from mosaics with different amounts of mutant tissue. — We prove here that for mosaics with an arbitrary fraction of mutant tissue (m), the largest frequency of separation that can occur is 2m. We prove that sturts underestimate actual distance on the blastoderm by a factor of r/m, where r is the radius of the mutant patch, and that sturts give no information on distances greater than 2r. This, and not double crossing over, is the reason for the nonadditivity of sturts and the shrinking of large distances in sturt measures. Sturtoids overestimate distances by a factor of 1/(2r) and also give no information on distances over 2r. This paper gives formulae for correctly estimating distance when using a collection of mosaics with varying amounts of mutant tissue. We also describe the nature of the errors introduced by convoluted or elongate mosaic boundaries and by multiple mosaic patches.

In fate mapping by the use of mosaics, the embryos made are composed of two genotypes, denoted mutant and normal or male and female. In a series of embryos, the dividing line between the two genotypes is randomly oriented. The embryos are allowed to develop and the adult structures are scored for genotype. The experimenter then tabulates the fraction of mosaics in which two structures are of different genotypes. This fraction may be called the frequency of separation. The adult structures descend from embryonic anlage that occupy particular sites on the blastoderm. "The further apart any two blastoderm sites are, the

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greater is the probability that the randomly oriented boundary in a mosaic blastula will pass between them" (Hotta and Benz 1972). It seems reasonable that the frequency of separation should increase monotonically with distance between anlage on the blastoderm. However, this has never been proven and no theory relating the frequency of separation to the actual distance on the blastoderm has been put forward. Authors have avoided this problem by calling the frequency of separation a “distance” and calling the units of this distance “sturts.” There has been little work to specify further the relation between “sturt distances” and actual distances on the blastoderm (see Lawrence and Morata 1977). The current paper considers this relationship explicitly.

Some problems with using sturts as distances have arisen. First, if the mosaic boundary actually divides the blastoderm into two equal halves, structures at opposite poles should always be of opposite genotype. They should be 1.00 sturt apart. However, the maximum sturt distance found between structures is only 0.50 to 0.60 sturts (e.g., Garcia-Bellido and Merriam 1969; Janning 1978). Second, sturt distances are found to be not additive. When a map is drawn, the sum of the short distances separating nearby structures between two distant structures is always more than the directly-measured frequency of separation between the distant structures.

This nonadditive nature (or in mathematical terms, nonlinearity) of sturts has been explained as being due to the fact that “the majority of mosaic boundary lines are highly irregular rather than straight lines” (Garcia-Bellido and Merriam 1969). It is argued that, “with large distances the probability increases that a boundary, having once intersected the line connecting the two sites, will cross back again, and both sites end up with the same genotype. This decreases the apparent value of large distances in analogy to the effect of double crossing over in genetic mapping” (Hotta and Benz 1972).

On further examination, this analogy to genetic mapping is not very convincing. If mosaic boundaries were straight lines, a given line would either cut between two sites once or not at all. Consider two points \( i \) and \( j \) and two straight mosaic boundary lines (Figure 1A). Line 1 cuts between \( i \) and \( j \), while line 2 does not. If the lines were irregular (Figure 1B), line 1 might take a jog and not cut (or cut twice) between \( i \) and \( j \). However, it is also possible that a line like line 2, which should not cut between \( i \) and \( j \), might take a bend of the same magnitude and cut between them. It appears that both types of event might be equally likely. Thus, there is no a priori reason to believe that the irregularity of mosaic borders either increases or decreases sturt distances. We will show that irregularity has its effects primarily at short distances and that the apparent decrease of long sturt distances has a completely different explanation.

**RELATION OF STURTS TO DISTANCE: IDEAL CASE**

We want to find the relation between the frequency of separation (sturt distance) of two structures by a mosaic boundary line and their actual distance

*For mathematical convenience we express sturt distances as a fraction ranging from 0 to 1, rather than the more usual convention of defining sturts to range from 0 to 100 as a percentage.*
of separation on the blastoderm surface. We consider the egg to be a surface in three dimensions and the mosaic boundary line to be a closed curve dividing the surface into two parts.

We would like to be able to calculate the probability that a randomly placed dividing line runs between two arbitrary points as a function of the distance between the two points. Problems of this nature are in the realm of "integral geometry" (see Kendall and Moran 1963) and are soluble in closed form only in very special cases. The problem, however, is easily solved for spherical surfaces where the boundary line is a great circle that divides the embryo into two hemispheres of different genotype (i.e., male and female, etc.).

Consider two points, \( i \) and \( j \), on the surface of a sphere (Figure 2). Any two points define a great circle. Draw the great circle defined by \( i \) and \( j \). Consider any half (\( II \)) of this great circle that includes \( i \) and \( j \). Any other great circle—for
instance, the mosaic boundary line—on the sphere intersects this half-circle exactly once. The probability that a randomly chosen great circle cuts across II in a given segment, ΔII, is the same for any location of ΔII and is proportional to the length of ΔII. The probability is thus ΔII/total length of II. Let ΔII be \( ij \), the distance between points \( i \) and \( j \). Then the probability, \( s \), is \( ij/\text{total length of II} \) or \( ij/\pi R \), where \( R \) is the radius of the sphere.

\[
s = \frac{ij}{\pi R}
\]  

(1)

This probability is the frequency with which the two points \( i \) and \( j \) will be separated by genotype: in other words, \( s \) is the frequency of separation or stunt distance. Thus, in this ideal case, sturts are a linear measure of the actual distance between \( i \) and \( j \) measured along the great circle between them. The proportionality constant is \( \pi R \).

Note that \( s \) is always a probability and therefore a dimensionless number running from 0 to 1. Equations in this paper are derived to be valid when using any units to measure distance. However, in fate mapping, it is conventional to scale distances so that the half-circumference of the blastoderm is equal to one unit (or 100 percentage points). If distance is measured in units where the half-circumference (\( \pi R \)) is one unit, then \( \pi R = 1 \) and equation (1) becomes:

\[
s = \frac{ij}{\pi R}
\]  

(1')

Equations valid only in these normalized length units will be numbered with primes.

**RELATION OF STURTS TO DISTANCE: MALENESS ≠ ONE HALF**

The linearity of sturts with physical distance breaks down in situations other than the ideal case. A common situation is one in which the patch of mutant (or male) tissue is not, in each mosaic, half of the whole (Kankel and Hall 1976). A large variety of methods can be used to create genetic mosaics (Hall, Gelbart and Kankel 1976). Each of these methods causes chromosome loss or rearrangement at different times in development. Thus, each method produces a different range and average proportion of normal and mutant tissue. In accord with the terminology appropriate for gynandromorphs, we will use the term male to denote tissue of one genotype in the mosaic, and female to denote the other genotype. Even in cases where the average maleness in a collection of mosaics is about one half, the collection will be made up of some individuals that are more than one half male and some that are less than one half male.

Consider a set of mosaics all of which have the same size of male patch, but the patch is less than half of the total. Let \( m \) be the fraction of the total blastoderm surface area which is male (fractional area = \( m \)). Since the patch is placed randomly on the blastoderm, the probability that any given structure is male is then also \( m \). Let \( r \) be the radius of the maleness patch (see Figure 3A).
If \( i \) and \( j \) have zero distance between them, they are either both in or both outside the patch. So there is a zero probability that they are of different genotype. Their sturt distance and their actual distance are both zero.

If \( i \) and \( j \) are greater than \( 2r \) apart, it is impossible for both to be in the male patch; if \( i \) is in then \( j \) is out and vice versa. The probability that \( i \) is in, is \( m \), and the probability that \( j \) is in, is \( m \). Since they are mutually exclusive events, the probability that either is in is \( 2m \). For the two points to be of opposite genotype, one must be in the male patch, so the sturt distance between the two points is also \( 2m \). Thus, when the physical distance between two points is \( 2r \) or greater, the sturt distance is a constant \( 2m \). We can thus draw a rough graph of the relationship between sturt distance and physical distance (Figure 3B). Appendix 1 shows that between 0 and \( 2r \) the graph is very nearly linear; it is drawn linearly in Figure 3B. The equation for this graph is

\[
\begin{align*}
    s &= (m/r) \times (ij) & 0 \leq ij \leq 2r \\
    s &= 2m & ij \geq 2r
\end{align*}
\] (2)
where $ij$ is the physical distance from $i$ to $j$, $s$ is the frequency of separation (or stunt distance) and $r$ is the radius of the maleness patch. The equation can be rearranged to solve for $ij$

$$ij = (r/m)\cdot(s) \quad 0 \leq s \leq 2m$$

$$ij \geq 2r \quad s = 2m$$

Thus, using a mosaic generating system that creates maleness patches of size $m$, the maximum distance that can be measured by using sturts is $2r$.

In the above discussion, male patches less than half the embryo were discussed. Small male patches occur when chromosome loss happens not at the first division but at a later division. Repeated chromosome loss can result in male patches greater than half the embryo. In this case, the female part of the embryo ($f$) is less than one half. By the same arguments, formulae 2 and 3 can be derived, with $m$ replaced by $f$. Similarly, the maximum frequency of separation that can occur is $2f$, and the maximum measurable distance is $2r$, where $r$ is now the radius of the female patch. Figure 3C shows the maximum frequency of separation and the maximum measurable distance as a function of the size of the maleness patch.

**Proportionality constant:** To get an idea of the numerical values involved in equation (3), the proportionality constant $r/m$ must be determined. The proportionality constant is the ratio of the radius of the male patch, $r$, to the fractional area of the patch, $m$. Idealizing the blastoderm to a sphere and the maleness patch to a circular region on the sphere results in the following: The area of a circular patch of radius $r$, on a sphere of radius $R$ is

$$A_r = 2\pi R^2 \left[1 - \cos \frac{r}{R}\right]$$

The area of the sphere is:

$$A_s = 4\pi R^2$$

$m$ is the ratio of the two:

$$m = \frac{A_r}{A_s} = \frac{2\pi R^2 \left[1 - \cos \frac{r}{R}\right]}{4\pi R^2} = \frac{1}{2} \left[1 - \cos \frac{r}{R}\right]$$

Solving for $r$:

$$r = R \cos^{-1} \left[1 - 2m\right]$$

Using normalized length units: $\pi R = 1$ unit

$$R = 1/\pi$$ unit

and equation (4) becomes

$$r = \cos^{-1} \left[1 - 2m\right]/\pi$$

Finally:

$$r/m = \cos^{-1} \left[1 - 2m\right]/\pi m$$

This is a reasonably cumbersome formula, but $r/m$ can be easily tabulated for different values of $m$ (Table 1).

<table>
<thead>
<tr>
<th>$m$</th>
<th>0.1 or 0.9</th>
<th>0.2 or 0.8</th>
<th>0.25 or 0.75</th>
<th>0.3 or 0.7</th>
<th>0.4 or 0.6</th>
<th>0.5</th>
</tr>
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<tr>
<td>$r/m$</td>
<td>2.05</td>
<td>1.48</td>
<td>1.33</td>
<td>1.23</td>
<td>1.09</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Using the above values for $r/m$ in equation (3), $ij = (r/m) \cdot (s)$, we can see the relationship between stunt distances and physical distances on the blastoderm. For a given frequency of separation, $s$, we can calculate the physical distance. Using a maleness of 0.25 (chromosome loss at the second rather than the first division), Figure 4 shows the relationship between stunt distance and physical distance. Sturts underrate all distances out to $2r$ by the factor $r/m$ and then consider all distances greater than $2r$ to be at the same distance. Figure 5A shows the geometric distortion caused by using sturts to map distances. From any landmark, $i$, distances less than the diameter of the maleness patch are shrunk by $r/m$, distances greater than the maleness diameter are all collapsed to the rim of the map at a distance of $2m$, and the map has no distances larger than $2m$.

**Sturts:** Gelbart (1974) has suggested using a different mapping function when mosaics have a maleness different from one half. Various authors have used this function (Kannel and Hall 1976; Hall 1977; Wilson 1981). Gelbart (1974) divides the frequency of separation of two structures by the sum of the maleness of the two structures. In most cases the two structures have the same, or similar, malenesses, so his procedure essentially divides $s$ by $2m$. He calls the new unit a “sturtoid”. Gelbart (1974) gives no reason for this procedure, but calls it “a metric of more general utility.”

A graph of Gelbart’s function is shown in Figure 4. Sturtoids overstate all distances out to $2r$ by the factor $1/(2r)$ and then all structures with greater separation than the diameter of the maleness patch are placed 1 sturtoid (or 100 sturtoids, in percentage notation) apart. Figure 5B shows the geometric distortion caused by using sturtoids. Distances less than the diameter of the maleness patch are expanded, distances greater are all collapsed to the extreme rim of the map.

![Graph of stunt distance and sturtoid distance vs. real distance for mosaics with a maleness of 0.25.](image)
Nonadditivity of sturts: It is now clear that when mosaics with a maleness other than one half are used, sturts are not a linear measure over their whole range because long distances will be understated. Basically, this is because the largest frequency of separation that can occur is $2m$. When $m < \frac{1}{2}$, or $m > \frac{1}{2}$, sturts are not additive and the largest observed sturt distances will be less than 1.

For some sets of mosaics it is reported that the average maleness for any structure is near 50%. Unfortunately, these samples usually include many flies with maleness greater than one half and many with maleness less than one half. It was believed originally that if the surface of a particular mosaic fly showed a small percentage of maleness, the internal tissues would be mostly male and vice-versa. Thus, even though many flies in the sample did not look externally like a 50/50 mosaic, it was assumed that if internal structures were included, the fly would indeed be 50/50. KANKEL and HALL (1976) have shown that this is not true. In fact the degree of internal maleness is directly rather than inversely correlated with the degree of external maleness.

Almost all samples of mosaics probably include many individuals with maleness $\neq \frac{1}{2}$. It can be seen from the results of this paper that including in the sample a mosaic with maleness $> \frac{1}{2}$ does not compensate for including a mosaic with maleness $< \frac{1}{2}$. In fact, the distortion is in the same direction (shrinkage).
on both side of $m = \frac{1}{2}$ and becomes greater the further $m$ is from $\frac{1}{2}$. This is probably the fundamental reason why stunt distances have been found to be nonadditive and why the maximum empirically-observed stunt distances have been so much less than 1.

A procedure for mapping a collection of mosaics with varying degrees of maleness: Most collections of mosaics will include flies with varying degrees of maleness. Since the relationship between distance and frequency of separation is different for mosaics of different maleness, the flies cannot all be treated in the same way. Mosaics of different maleness have a different proportionality constant between distance and stunts, and a different maximum measurable distance. Thus, the mosaics should be sorted into groups with similar maleness and then the proper proportionality constant (Table 1) and maximum measurable distance should be applied to each group. Finally the results from the different maleness groups should be combined. This can be done by taking a weighted average of the distances, with the weighting factor being the number of flies in that class.

For each mosaic the proportion of its scored structures that are male should be noted. Since the maleness of unscored structures is likely to be highly correlated with the maleness of scored structures (KANKEL and HALL 1976), the latter will serve as an estimate for the whole fly. The mosaics may then be divided into five or so groups depending on their maleness. Flies with maleness $m$ and $1-m$ would be included in the same group; for example, $m = 0.1$ and $m = 0.9$ flies would be included in the same group.

For each group the fraction of mosaics, $s(m)$, in which two structures are of different genotype can be tabulated. We know that when the frequency of separation approaches $2m$, $s$ stops being a measure of the distance between the two points. Accordingly for points where $s(m)$ is close to $2m$, the contribution of that maleness group to the distance estimate must be ignored. The distance is related to the frequency of separation by equation (3)

$$ij = \frac{r}{m} \times s(m)$$

where the values of $r/m$ are those in Table 1. The distance estimate for each maleness group should be about the same. If there are slight differences, the data from the different maleness groups can be combined in a weighted average using the number of flies in that group as the weighting factor.

In different fate mapping experiments the relative location of the various landmarks remains relatively constant. However, the distances between landmarks are not very reproducible. Thus the distance from the palp to the first leg is shown on various maps as 22 sturtoids (HOTTA and BENZER 1972) to 66 sturtoids (HALL 1977, Figure 3, sum of pa to og1 and og1 to prc) to 93 sturtoids (KANKEL and HALL 1976, figure 10, sum of pa to slg and slg to prc). These different experiments use different mosaic generating systems; they probably have very different distributions of the sizes of maleness patches. Since this is likely to be the major source of error, use of the above procedure should make these maps
more uniform. We hope that standard maps of inter-landmark distances can now be prepared that will be accurate in any mosaic generating system.

EFFECT OF IRREGULAR BOUNDARIES

The boundaries of real mosaic patches are not smooth circles, but have quite irregular outlines (ZALOKAR, ERK and SANTAMARIA 1980). It is important to

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**Figure 6.**—Frequency of separation for mosaic patches with convoluted borders.

A. An extreme example of a mosaic patch with a convoluted border.

B. Line a: frequency of separation considering only those cases where one point is inside the circular envelope and one outside. Line a+b: frequency of separation also including cases where both points are inside the circular envelope.

C. Comparison of frequency of separation for the ideal (circular) case (solid line) and worst possible case (dashed line). The curve for any real mosaic must lie between the two lines.
know how irregularities affect the relationship between frequency of separation and distance. We will consider separately three forms of distortion. Starting from the ideal circular patch, we will first consider the effect of convolution of the border, where the border has a complex shape but where the envelope of the male patch is still basically circular (Figure 6A). Then we shall consider cases where the patch is no longer circular, but is elongate in any direction (Figure 7B). Finally, we will consider mosaics with several discrete patches. Most of the male patches shown histologically by Zalokar, Erk and Santamaria (1980) can be considered as a combination of these three distortions.

Convolution of the border: Consider a male patch with any irregular boundary that can be enclosed within a circular envelope (Figure 6A). In the worst possible case, the real boundary is so irregular that half the points within the limiting circle are of one genotype, and the other half are of the opposing genotype. The fractional area \( m' \) enclosed within the limiting circle (radius \( r' \)) will be twice the actual maleness \( m \). Also in the worst case, two points any distance apart within the envelope have an equal chance of being of the same or different genotype. Two points \( (i \) and \( j \) can be of different genotypes if a) one point is outside the limiting circle and the other point is in, or if b) both points are in the limiting circle.

\[
\frac{1}{2} \cdot \pi \cdot r'^2 = 2m 
\]

Figure 7.—Frequency of separation for mosaic patches with elongate borders.  
A. Circular patch of fractional area \( m \) and maximum dimension \( 2r \).  
B. Elongate patch of fractional area \( m \) and maximum dimension \( D \).  
C. Comparison of frequency of separation for circular patch (straight lines) and elongate patch (curving line).
a) By the same logic used to derive equation (2), the probability that one blastoderm point is in the limiting circle and one is out is

\[ p = \left( \frac{m'}{r'} \right) \cdot ij \text{ for } ij < 2r' \text{ and } p = 2m' \text{ for } ij \geq 2r' \]

However, with the irregular boundary, half the time the inside point will have the same genotype as the outside point. So the frequency of separation is half the above value:

\[ s = \left( \frac{m'}{2r'} \right) \cdot ij \text{ for } ij < 2r' \text{ and } s = \frac{2m'}{2} \text{ for } ij \geq 2r' \]

But since \( m'/2 = m \) and \( r' \) is approximately \( \sqrt{2r} \) (where \( r \) is the radius of a circle of fractional area \( m \); the exact value for \( r' \) may be found from equation 4'), we get

\[ s = \left( \frac{m}{\sqrt{2r}} \right) \cdot ij \text{ for } ij < 2\sqrt{2r} \text{ and } s = 2m \text{ for } ij \geq 2\sqrt{2r} \]

This equation is graphed in Figure 6B as line a.

b) If both points are in the limiting circle, given the worst case described above, half the time they will be of different genotype. If \( i \) and \( j \) are very close together, the probability that both points are in the limiting circle approaches \( m' \). The probability that they are of different genotype is \( m'/2 \) or \( m \); so at the left end of the graph (Figure 6B), case b adds \( m \) to the frequency of separation. If \( ij \) is \( > 2r' \), \( i \) and \( j \) cannot both be in, and case b adds nothing to the graph. It can be shown by a simple argument that between 0 and \( 2r' \), a linear rise for the frequency of separation is as good an approximation as it is in the ideal case (APPENDIX 2). The total frequency of separation is the line a+b in Figure 6B.

Figure 6C compares the frequency of separation (equation 2) for a circular patch of maleness \( m \) (solid line), and for the worst case, a convoluted patch of the same maleness (broken line). For all real patches on a mosaic, which will not be as extreme as the worst possible case, the graph of frequency of separation must run between the ideal and the worst possible case (i.e., in the cross-hatched area of Figure 6C).

Elongate patches: If a circular patch has a fractional area \( m \), then it has a maximum dimension of \( 2r \) (where \( r \) is its radius, Figure 7A). However, an elongate patch of the same area will have a longer maximum dimension, say \( D \), Figure 7B. Consider the case where \( ij \) is between \( 2r \) and \( D \). For a circular patch (diameter \( 2r \)), each time one point is in, the other must be out (Figure 3A) so the frequency of separation is the sum of the probabilities (\( m \)) that either is in. This sum is \( 2m \). For the elongate patch, the probability that either point is in is still \( m \); but, for some locations of the patch, when one point is in the other will also be in. So the frequency of separation at these distances is less than \( 2m \). Thus, two blastoderm points between \( 2r \) and \( D \) apart will have a lower frequency of separation in mosaics with elongate patches than in mosaics with circular patches.
When the blastoderm points are a short distance apart, elongation of the patch has the opposite effect. For a mutant patch of a given area, the boundary line with the smallest perimeter is a circle. The more elongate the patch (at constant \( m \)), the longer the border. The border can be intersected by \( ij \) only if one point falls within the patch, but in a zone no more than \( ij \) from the border. For more elongate patches, this zone takes up a larger fraction of the total patch area. Thus, if \( ij \) is short, it has a greater chance of intersecting the border in an elongate patch than in a circular patch. The exact quantitation of this effect is complex, but it can be shown* that in the case of the most elongate patches, in the limit of small \( m \), the frequency of separation approximates \( 2ij \) for small \( ij \). The curve at the left end of Figure 7C rises much more steeply for elongate patches than for circular patches.

*The most elongate patch is one which is an equatorial zone completely encircling the sphere. In the limit of small \( m \), both the northern and southern borders of the patch are nearly great circles. The probability of intersection of \( ij \) with a great circle is \( ij \) (equation 1'). As long as \( ij \) is less than the width of the zone, it can intersect the northern or southern borders of the zone, but not both. Thus the total probability of \( ij \) intersecting the border is \( 2ij \).
patches are convolute, the maximum addition to the frequency of separation is \( m \). The frequency of separation reaches its maximum at the maximum dimension of the largest male patch.

**Absolute limit of frequency of separation:** For any patch of fractional area \( m \), independent of its shape, the probability that one blastoderm point (say \( i \)) is in the patch is \( m \). The probability that another point (say \( j \)) is in the patch is also \( m \). The maximum frequency of separation occurs when these are mutually exclusive events, e.g., under conditions where if one point is in the other must be out. This happens, for instance, with circular patches when \( ij > 2r \). In these cases the frequency of separation is \( 2m \). Thus, the maximum frequency of separation for any two points and any patch shape is \( 2m \).

It can be seen that every distortion of the border acts primarily to increase the frequency of separation of the shorter distances, while it does not effect the longest distances. This is exactly the opposite conclusion to that previously drawn (Hotta and Benzer 1972). It also throws into doubt Flanagan's (1981) contention that "The excess of separation frequencies . . . affords a measure of the magnitude of ancestry variances." Much, if not all, of the excess must be due to irregular mosaic boundaries and can not be used as evidence for indeterminacy in the ancestry of adult structures.

The overall effect of irregular and noncircular borders is to make fate mapping less accurate. A distorted border flattens the relationship between frequency of separation and distance, making all distances appear to be more nearly the same.

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**LITERATURE CITED**


We wish to calculate the probability that two blastoderm locations will be of different genotype in a series of mosaics of arbitrary maleness as a function of the distance between the points. Consider a blastoderm with two points, $i$ and $j$, on it. A patch of male cells is placed randomly on the blastoderm. The line between $i$ and $j$ can intersect the male patch in four ways (see Figure 8A);

**Figure 8.**—A. A patch of male cells with different possible positions for two anlage ($i$ and $j$).
B. A spherical blastoderm with a male patch on it.

I. $j$ can be in and $i$ out;
II. $i$ can be in and $j$ out;
III. $i$ and $j$ can both be in;
IV. $i$ and $j$ can bracket the patch but both $i$ and $j$ are out.

The frequency of separation of the two points is the probability that case I or case II occurs.
We would like to calculate this probability, but the relation between the distance $i j$ and the frequency of separation is not quite linear and can not be written down as an analytic function. However, given a simple geometry, a slightly different probability can be calculated that does increase linearly with the distance $i j$. The difference between the two probabilities is always small compared to the probability of I or II, so we can show that the probability that I or II occurs is nearly linear with respect to $i j$.

Let us assume that the blastoderm is a sphere (radius $R$) and that the male patch is a circular region (radius $r$) on the sphere (Figure 8B). The two points $i$ and $j$ define a great circle on the sphere. Let this great circle be the equator. The center of the male patch may lie anywhere on the sphere. If the center of the male patch lies within a distance $r$ above (or below) the equator, the equator will intersect the male patch. We only have to consider one hemisphere because the probabilities are the same for the other hemisphere. The probability that the center of the patch lies in a zone $d h$ wide, centered at a distance $h$ from the equator, is equal to the area of the zone, divided by the area of the hemisphere. The area of the zone is equal to $d h$ times the circumference of the latitude circle at the latitude $h/R$. This circumference is $2 \pi R \cos h/R$ (Gellert et al. 1977). So the probability that the center of the male patch is $h$ to $h + d h$ above the equator is:

$$\frac{\text{area of zone } d h \text{ wide}}{\text{area of hemisphere}} = \frac{\text{circumference}(h) \, d h}{2 \pi R^2} = \frac{2 \pi R \cos(h/R) \, d h}{2 \pi R^2} = \frac{1}{R} \cos \frac{h}{R} \, d h \quad (5)$$

for $0 \leq h \leq r$

The equator intersects the male patch when $h$ is between 0 and $r$. Given this intersection, what is the probability that the segment $i j$ of the equator intersects the male patch in configurations I or II of Figure 8A. The position of the segment $i j$ is defined by the position of its center. For case I point $j$ will be in, and $i$ out, if the center of $i j$ lies anywhere from $i j/2$ to the left of the boundary of the male patch to a distance $i j/2$ inside the patch (see Figure 9, left side).

**Figure 9.**—A patch of male cells on a blastoderm. The lines denote the range of positions which the center of $i j$ can take and still have $i$ be of different genotype from $j$. The length of each line is $i j$. At any one latitude, there are two lines each of length $i j$. At the extreme southern edge of the male patch the two lines of length $i j$ overlap.
The center of \(ij\) can thus range over a total distance of \(ij\) for case I. Similarly for case II the center of \(ij\) can range over a total distance of \(ij\), but now on the right side of the male patch. Thus, if the center of \(ij\) lies within these two intervals, whose total length is \(2ij\), then \(i\) and \(j\) will be of different genotype.

This situation holds except when the center of the male patch falls far north of the equator so that the equator intersects the south end of the male patch. Here the left \(ij\) segment will overlap with the right \(ij\) segment. If we still allow the center of \(ij\) to range over a distance of \(2ij\), we include some cases where \(i\) and \(j\) are not separated by genotype. If the center sits in the vertically-hatched region (Figure 9), \(i\) will sit outside the male patch to the left and \(j\) will sit outside the male patch to the right (configuration IV). That is, the segment \(ij\) will bracket the male patch leaving both \(i\) and \(j\) outside. If the center of \(ij\) sits in the horizontally-hatched region, then \(i\) and \(j\) will both be to the left of the male patch, or both to the right.

The probability that we shall calculate is greater than the probability of separation because of the inclusion of the hatched area. The hatched area will always represent a small fraction of the total area in which the center of \(ij\) can sit. It can also be seen that the hatched area decreases as \(ij\) gets smaller.

The probability that the center of \(ij\) sits in a particular segment of the equator is the ratio of the length of the segment to the length of the equator. In the situation discussed above that ratio is:

\[
\frac{2ij}{2\pi R} = \frac{ij}{\pi R}
\]

The probability that a randomly placed male patch intersects the segment \(ij\) in the configurations of Figure 9 is the product of the probability that the equator intercepts the patch (equation 5) times the probability that the intersection includes the segment \(ij\) in the manner of Figure 9 (equation 6). This probability is:

\[
p = \int_{h=0}^{h=\pi} \frac{ij}{\pi R} \times \frac{1}{R} \cos \frac{h}{R} dh
\]

Thus

\[
p = \frac{ij}{\pi R} \int_{h=0}^{h=\pi} \cos \frac{h}{R} dh = \frac{ij}{\pi R^2} \times R \sin \frac{h}{R} \bigg|_{h=0}^{h=\pi} = \frac{ij}{\pi R} \sin \frac{r}{R}
\]

This equation is linear in \(ij\) and the constant of proportionality,

\[
\frac{1}{\pi R} \sin \frac{r}{R},
\]

depends on the radius of the maleness patch. If we want to use units such that the half circumference of the sphere is one unit, then \(\pi R = 1\) and the probability becomes

\[
p = ij \sin \pi r
\]

For any given size maleness patch we can graph equation 7' (Figure 10). We know that \(p\) is always greater than the frequency of separation, \(s\), because of the inclusion of the hatched areas. Thus the graph of \(p\), as calculated by equation 7, is an upper bound for the graph of \(s\). We know that \(s\) has a maximum of \(2m\) when \(ij = 2r\). This places point \(a\) on the graph for \(s\). We know that the excess (hatched area) of equation 7 over \(s\) is greatest when \(ij\) is greatest. Thus \(s\) is always below equation 7, but never further below than at point \(a\). This fixes a lower limit for \(s\), drawn as the dotted line in Figure 10. We also know that the frequency of separation is 0 when \(ij\) is 0. This fixes the low point of \(s\) at the origin. Thus \(s\) starts at the origin,
ends at a and lies within the upper and lower bounds shown in Figure 10. Figure 10 has been drawn to scale for a maleness of 0.25. If the maleness is greater than this, the difference between $s$ and equation 7 is even less. It is clear that we can approximate $s$ by a straight line without being too far wrong. The equation of that straight line is

$$s = \frac{m}{T} \cdot ij\quad(2)$$

**APPENDIX 2**

**LINEARITY OF FREQUENCY OF SEPARATION: CONVOLUTED BORDER**

The probability that one point is in the limiting circle and the other is out is:

$$\frac{m' \cdot r'}{r} \cdot ij\quad(a)$$

This is the formula whose near linearity is shown in Appendix 1. In half these cases $i$ is in the limiting circle and $j$ is out. The probability of this is:

$$\frac{1}{2} \left(\frac{m' \cdot r'}{r'}\right) \cdot ij\quad(b)$$

The probability that $i$ is in the limiting circle is:

$$m'\quad(c)$$

When $i$ is in, $j$ must be either in or out. The probability that both are in is:

$$\frac{1}{2} \left(\frac{m' \cdot r'}{r'}\right) \cdot ij\quad(d)$$

The frequency of separation is half the probability that one point is in and the other point is out (a), plus the probability that both are in (d):

$$s = \frac{1}{2} \left(\frac{m' \cdot r'}{r'}\right) \cdot ij + \frac{1}{2} \left(\frac{m' \cdot r'}{r'}\right) \cdot ij = m' + \frac{1}{2} \left(\frac{m' \cdot r'}{r'}\right) \cdot ij$$

This equation is just as linear as (a).