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A Method for Estimating the Number of Blastoderm Cells Which Give Rise to *Drosophila* Imaginal Discs

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Summary. A mathematical method for calculating the number of blastoderm cells whose descendants form the various imaginal discs is described. The method differs from available approaches in two respects: (1) It is based only upon the frequency of mosaicism of the adult derivatives of a given imaginal disc and ignores the relative surface area of the two genetically marked cell populations which comprise these derivatives. (2) The method estimates the average number of cells at the blastoderm stage which give rise to a particular imaginal disc and not at the developmental stage at which restriction of the pool of cells which will form this imaginal disc occurs. Despite their methodological differences the estimates obtained from this method and from other approaches are of the same order of magnitude and thus provide further support to the currently available estimates and to the notion that restriction of whole imaginal discs occurs at the blastoderm stage. The proposed method also provides a quantitative approximation of the non-linear relationship that exists between the frequencies of mosaicism of different imaginal discs and the number of cells which comprise these discs.

Introduction

The imaginal discs of *Drosophila* result from proliferation of clusters of cells which are set aside early in embryogenesis (see Ursprung and Nöthiger, 1972, for a review). It is of considerable interest to determine the number of blastoderm cells from which particular imaginal or larval structures arise. Unfortunately, since at the blastoderm stage the cells which give rise to the various imaginal discs are cytologically indistinguishable from the surrounding cells, no direct method exists to determine their number (Gehring and Nöthiger 1973). The only available relevant information is based on indirect estimates which are derived from studies of genetic mosaics.

In genetic mosaics which are the result of X-chromosome elimination (gynandromorphs), it is found that a certain body part which arises from a single imaginal disc may itself be occasionally mosaic; i.e., it is constituted of two populations of cells of unlike genetic constitution (Sturtevant 1929). In gynandromorphs, where elimination of an X-chromosome usually occurs during the early cleavage divisions (Sturtevant 1929; Garcia-Bellido and Merriam 1969; Hotta and Benzer 1972), this mosaicism can be interpreted by assuming that at the time these imaginal disc precursors become delineated from other epidermal cells, they already consist of a genetically mixed population of cells (Stern 1940).

Methods exist that make it possible to estimate the number of primitive cells whose descendants form a particular adult body structure, i.e., the number of cells at the stage of embryonic development when restriction (or allocation) of the pool of cells which will form the body structure occurs. These methods are based on measurements of the smallest fraction of a complete mosaic structure of one genotype in a significant number of gynandromorphs. If it is assumed that the smallest fraction corresponds to the derivatives of a single primitive cell, then the reciprocal of this fraction provides an estimate of the number of primitive cells (Stern 1940). Thus, Stern's data consisted of 26 gynandromorphs with a mosaic mesonotum. In 11 out of these 26 gynandromorphs, 1/8 of the mesonotal surface area consisted of cells of one genetic constitution (X0 or XX) and 7/8 of the mesonotal surface area consisted of cells of the other genetic constitution. He concluded therefore that the minimal number of primitive nuclei which initiate the mesonotum is 8. The accuracy of such estimates rests upon various simplifying assumptions (see Gehring and Nöthiger 1973, p. 219, for a review). Hence, as Stern himself realized, the meth-

od gives only an estimate of the order of magnitude involved.

In order to circumvent some of the difficulties inherent in Stern's procedure, other approaches have been used. Garcia-Bellido and Merriam (1969) based their estimates on scoring definite cuticular landmarks in the adult (rather than total surface area). Postlethwait and Schneiderman (1971) estimated the number of cells giving rise to the antennal disc by a modification of Nance's (1964) method. These three approaches are based on the assumption that all the cells which initiate an imaginal disc have similar division rates, and hence that their descendants are equally represented in the adult cuticular surface. Evidence exists, however, which indicates that different regions of a disc may have different proliferation rates (Gehring and Nöthiger 1973; Postlethwait and Schneiderman 1971). All three approaches also encounter the technical difficulty of determining accurately in each case what fraction of the particular mosaic structure is of each genotype.

In addition to the difficulties inherent in these fractional methods they provide no information regarding the developmental stage at which restriction of the pool of cells which will form a particular imaginal disc occurs. The fractional approach shows, for example, that the minimal number of cells which initiate the fourth half-tergite is 8 but it does not show at what stage this initiation, i.e., restriction from further supply of cells, occurs. The most likely stage at which restriction occurs is the blastoderm stage (e.g. Bryant and Schneiderman 1969), but critical experimental evidence is yet lacking. It seems desirable therefore to devise a method that uses the frequency of mosaicism of a disc as its starting point rather than the fraction of genetically marked cells and that determines the number of blastoderm cells which give rise to a particular imaginal disc rather than the number of cells at the developmental stage at which restriction occurs. In addition to providing a direct estimate of the number of blastoderm cells, such a method may provide some insight concerning the developmental stage at which restriction occurs and concerning the accuracy of available estimates of the number of primitive cells.

Sturtevant (1929) recorded frequencies of mosaicism of various cuticular structures in *Drosophila simulans* gynandromorphs and suggested that the observed frequency differences are probably a function of the numbers of blastoderm cells from which the particular imaginal discs are derived. Thus, the mesothoracic disc "is most often mixed. This presumably means that it comes from a larger number of blastoderm cells than do the other discs" (Sturtevant 1929). It is the purpose of this note to give a more quantitative expression to Sturtevant's original insight.

The Method

1. The relationship between blastoderm cell number and mosaic frequency

It is clear that the larger the number (N) of blastoderm cells which give rise to a given region in the adult, the greater the probability (P) that this region will contain cells of unlike constitution. Now, P is an experimental datum; for instance, the right or left fourth half-tergites were mosaic in 15 out of a total of 94 gynandromorphs scored (Garcia-Bellido and Merriam 1971). Since the fly is bilaterally symmetrical, it may be approximated that the probability that a gynandromorph will have a mosaic right half-tergite is $15/2 \times 94$ or $P = 0.08$. Note that P is roughly equal to the frequency of mosaicism of a certain region but that it is derived in a different manner; one is interested here in computing the probability that one bilateral region is mosaic.

The purpose of this paper is to obtain a function which will make it possible to compute the number (N) of blastoderm cells which give rise to a particular adult body region from a given P. An indirect approach to this problem is made if it is assumed that:

1. At the end of blastoderm formation, the cells on the blastoderm occupy all the available space in the cortical layer and are evenly spaced (Sonnenblick 1950).

2. There are at this stage some 3400 cells on the blastoderm surface (Sonnenblick 1950).

3. The blastoderm surface may be idealized as a sphere on which "great circles" (mosaic boundaries) are drawn at random and each such drawing event is independent of the others (Parks 1936; Garcia-Bellido and Merriam 1969; Hotta and Benzer 1972).

4. The great majority of gynandromorphs are the result of X-chromosome loss during the first mitotic

division so that the mosaic patterns observed may be regarded as the "result" of drawing great circles on a sphere (Hotta and Benzer 1972).

5. The cells which give rise to a given post-embryonic structure occupy a circular region on the blastoderm surface.

6. When the great circle (mosaic boundary) intersects a region, this results in cells of unlike constitution, i.e., a mosaic structure.

Since the cells on the blastoderm surface are evenly spaced, the following relationship is roughly correct:

$$\frac{\text{surface area of region of blastoderm which gives rise to a certain imaginal disc}}{\text{total surface area of blastoderm}} = \frac{\text{N(number of blastoderm cells in this region)}}{\text{3400(total number of cells on blastoderm surface)}}$$

It follows that in order to obtain an estimate of N, one merely has to determine the ratio between the area which will give rise to a single unilateral region to the total surface area of the egg, given the probability (P) that a randomly drawn great circle intersects this region. The following section will present a mathematical solution to this problem.

2. Derivation of the function relating disc size and mosaic frequency

Given a sphere of radius R and a circular region on the sphere, we wish to find the probability P that a "randomly drawn great circle" intersects the given circular region. The relation obtained between P and the area of the circular region will enable us to find the area, given P.

The following definitions and conventions will be used:

A "great circle" is any circle drawn on the surface of the sphere which divides the sphere exactly in half. The center of each such circle is the center of the sphere, the radius is R, and the circumference is $2\pi R$ units.

When we say a great circle is drawn "at random" on the surface of the sphere, we mean that each such circle is as likely as any other to be the one drawn.

From now on, all points we talk about will lie on the surface of the sphere. The distance between two points will be measured along a great circle joining the points.

In the context of points and distances on the surface of the sphere, a great circle can be thought of as the set of points a distance $\pi R/2$ units from some fixed point on the sphere. We shall also be considering smaller circles on the surface of the sphere. A "circle of radius r" is the set of all points a distance r units from some point c. The point c is called the "center" of the circle. A "circular region" of radius r is the interior of such a circle; that is, the set of all points a distance less than r units from some center c.

Let A be a circular region of radius $r < \pi R/2$. We are interested in the probability P that a randomly drawn great circle intersects A.

Remark: We make the restriction $r < \pi R/2$ in order to make A smaller than a hemisphere. If A were a hemisphere or a larger region, then the probability would be 1 that a randomly drawn great circle would intersect A.

The probability P is the same as the probability that a randomly drawn circle of radius r intersects a fixed great circle. Selecting a circle of radius r at random is equivalent to selecting the center of such a circle at random. This is of use to us, since we know the probabilities associated with picking a point at random. The probability that a randomly chosen point lies within a given region is the ratio of the area of the region to the area of the sphere.

Let T be the set of points which are centers of circles of radius r intersecting a given great circle. Then T is the set of points a distance less than r units from the fixed great circle. It can be pictured as a band of width 2r about the great circle. As we noted, P is the probability that a randomly chosen point lies in T. Hence, P = area of T/area of the sphere = area of $T/4\pi R^2$.

Since T is a band about the sphere, the part of the sphere not contained in T consists of two "caps": circular regions on opposite sides of the sphere, each of radius $\pi R/2 - r$.

The surface area of a circular region of radius a on a sphere of radius R is $2\pi R^2(1 - \cos a/R)$. (See, for example, the CRC Handbook of Tables for Mathematics, 1975, p.17.) Thus the area of T is $4\pi R^2 - 2[2\pi R^2(1 - \cos(\frac{\pi R/2 - r}{R}))] = [4\pi R^2 \cos(\frac{\pi}{2} - \frac{r}{R})] = 4\pi R^2$

Table 1. Comparison of Independently Obtained Pairs of Estimates for the Number of Primitive and Blastoderm Cells

Region	P (frequency of mosaicism in this region)	Number of primitive cells obtained by fractional methods	Authors	Number of blastoderm cells obtained by Eq.(2)
Wing	0.180	30	Ripoll 1971	28
Mesonotum	0.115	17	Ripoll 1971	11
Mesothoracic disc	0.19[a]	40	Ripoll 1971	31
Fourth half-tergite	0.08	8	Garcia-Bellido and Merriam 1971	5
Antenna	0.180	8	Postlethwait and Schneiderman 1971	28
Eye-antennal disc	0.238	23	Garcia-Bellido and Merriam 1969	49

[a] The published value of 0.294 is an error (Ripoll; personal communication).

sin r/R. Then since P = area of $T/4\pi R^2$, we have P = sin r/R.

Our aim was to find a relation between P and the ratio of the area of the given circular region A to the area of the sphere. The area of A is $2\pi R^2(1-\cos r/R)$. Since P = sin r/R, we have $\cos r/R = \sqrt{1-P^2}$. Thus area of $A/4\pi R^2 = \frac{1}{2}\left(1-\sqrt{1-P^2}\right)$.

3. Comparison of estimates of primitve cell numbers obtained by fractional methods to estimates of blastoderm cell numbers obtained by the mosaic frequency method

It follows from the preceding analysis that the number of blastoderm cells (N) which give rise to a particular region may be estimated from the following equation:

$$N = \left[1/2\left(1-\sqrt{1-P^2}\right)\right] \quad 3400 \qquad (1)$$

or

$$N = 1700\left(1-\sqrt{1-P^2}\right) \qquad (2)$$

Table 1 provides a comparison of the estimates of blastoderm cells obtained by means of Eq.(2) to estimates of primitive cells obtained by fractional approaches. The value of Garcia-Bellido and Merriam (1969) is based on *Drosophila simulans* gynandromorphs; the remaining estimates are based on gynandromorphs of the sibling species *D. melanogaster*. Given the fact that both types of approaches rest upon imprecise assumptions, the general agreement between any pair of estimates lends support to the notion that the number of primitive cells is equal to the number of blastoderm cells which give rise to the same region and hence that restriction indeed occurs at this stage. It appears then that N values of the same order of magnitude are obtained with either approach. The only significant differences involve estimates of the antenna and eye-antennal disc. As we shall see in the following discussion, these differences may be explained either by the assumption that restriction does not occur at the blastoderm stage or by the more plausible assumption that these differences are due to the inherent inaccuracies of both the mosaic frequency method and the fractional method.

Discussion

It is obvious that the mosaic frequency method is based upon idealizations of biological realities; the egg, for instance, is not a sphere. Another example concerns the assumption that the mosaic boundary is

a straight line; the actual boundary is almost certainly quite convoluted. Also, some mixing of nuclei probably occurs before blastoderm formation; this may result in a wiggly boundary on the blastoderm surface and would tend to increase the frequency of intradisc mosaicism. We believe, however, that most of the underlying explicit or implicit assumptions made throughout the development of this method are not crucial to its validity. Two assumptions are controversial and involve some uncertainties and must therefore be considered here:

(a) Although the method provides a reliable estimate of the fraction of the blastoderm which is destined to produce a particular disc, the conversion of this fraction into cell number depends on an accurate estimate of the total blastoderm cell number. Throughout this report it was assumed that this number is roughly equal to 3400 (Sonnenblick 1950). Recently however, Zalokar (1975) has claimed that the total cell number at the time of blastoderm formation may be as high as 6400. The estimates derived from Eq (2) should be regarded therefore as tentative and should be modified if Sonnenblick's widely accepted estimate of the total cell number is too low. It should be stressed that the important feature of the mosaic frequency method is that it enables one to estimate the number of cells that comprise the blastoderm precursors of a given imaginal disc, given the experimentally ascertainable values of the frequency of mosaicism of this disc and of the total blastoderm cell number.

(b) The mosaic frequency method refers exclusively to the blastoderm embryonic stage; moreover, this is the only stage to which it can be meaningfully applied. It should be strongly emphasized, however, that this method is not committed to the controversial suggestion (cf., Bryant and Schneiderman, p. 285 1969; Wieschaus and Gehring 1975) that determination (or even restriction) of a particular group of adjacent cells to form a particular imaginal disc occurs at this stage. All that is needed for the method to work is that a correlation exists between the position of a group of cells on the blastoderm surface and the position of their descendants in the adult and that no significant mixing of nuclei occurs after the blastoderm stage. The method merely provides estimates of the average number of adjacent cells on the blastoderm surface whose descendants will form one particular disc. Thus for example, the method indicates that if the frequency of mosaicism of the fourth half-tergite is 0.08 and if the total cell number on the blastoderm surface is 3400, then the average number of cells which will take part in the formation of the adult fourth half-tergite is approximately 5. If the total cell number is 6400, then the number is approximately 9. Both methods say nothing about the time in development at which determination of whole discs occur, i.e., the time at which a cell is irreversibly established to become an indeterminate part of a specific disc. The mosaic frequency method approximates the average number of blastoderm cells that will become involved in the formation of the disc; the fractional approaches approximate the number of cells at the developmental stage at which the precursors of the imaginal discs are isolated from further supply of cells. It follows that the estimates of the two approaches are comparable only insofar as these two stages of development are identical. If they are not identical then the two approaches estimate the number of cells which give rise to a particular imaginal disc at two different stages of embryonic development and hence each approach may be valid and yet arrive at a different estimate. Since restriction of the pool of cells which will form a particular disc probably does occur at the blastoderm stage, a comparison of pairs of values obtained by these two approaches was attempted (Table 1). The fact that the values derived from the mosaic frequency method are similar to the values derived from fractional approaches suggests that these two developmental stages may be identical. However, the reader should bear in mind that, strictly speaking, the proposed method only attempts to estimate the number of blastoderm cells which give rise to particular discs and that equating this number with the number of cells at the developmental stage at which restriction occurs must await further experimentation.

The correspondence between values obtained by means of the mosaic frequency method to values obtained by means of fractional methods lends support to the notion that P is an important parameter in determining the number of primitive cells and should not be ignored. When there is a discrepancy between

these methods, a reexamination of the reported values may be necessary. For instance, the reported frequencies of mosaicism for the wing and antenna are equal (Table 1). This fact suggests that they originate from a similar number of cells. Nevertheless, the antenna anlage was estimated to consist of 9 cells and the wing anlage of 30. We feel that the former estimate is probably marred by the technical difficulties involved in determining the relative size of the two genetically marked cell populations in the antenna and hence, that it should be revised.

The mosaic frequency method makes possible a quantitative determination of the relative numbers of blastoderm cells which give rise to different imaginal discs. Garcia-Bellido and Merriam's (1969) modification of Stern's method, for example, led them to the conclusion that the number of cells in the mesonotal anlage and the eye-antennal anlage is roughly equal. These workers felt that this was in contradiction with their data that the eye-antennal disc is mosaic approximately twice as often as the mesothoracic disc. They suggested therefore that the eye-antennal anlage contains twice as many cells as the mesothoracic anlage. Our alternative approach suggests that the frequency of mosaicism of a structure is indeed related to the number of blastoderm cells. Moreover, the mosaic frequency method shows that the relationship between the frequency of mosaicism and the number of blastoderm cells is not a simple linear relationship (Garcia-Bellido and Merriam 1969), but rather, a more complicated relationship which is probably approximated by Eq.(2) above.

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