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On the dispersion of imaginal progenitor cells in the *Drosophila* blastoderm

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SUMMARY

The number of blastoderm cells in *Drosophila* whose descendants form adult structures has frequently been estimated from genetic mosaics. Data from somatic recombination (method I) and gynandromorph (method II) mosaics both yield very low estimates, e.g. about 10–20 progenitor cells for the eye and antenna, wing or leg.

In gynandromorphs the mosaic dividing line has a random orientation on the blastoderm. In the 6000 cell blastoderm it should be very unlikely that the mosaic dividing line passes through any small patch of only 10–20 cells. Yet it has been reported that 10–25 % of eye/antenna, wing or leg disks in gynandromorphs are mosaic. Thus the frequency of mosaicism data seems to be in contradiction to the progenitor population estimates. Similar discrepancies are found in the data for other adult structures.

In this paper we derive a formula for estimating the number of cells in a blastoderm patch from the frequency with which the gynandromorph dividing line passes through it (method III). In a second method (method IV) we use the maximum distances inside the progenitor areas on a fate map to estimate the progenitor patch size. These two estimates agree closely with each other. We find, e.g. that 50–100 cells are in the patches from which the eye/antenna, wing or leg disks derive.

We examine a number of possible explanations for why the first two estimates are so much smaller than the last two. The former estimates refer to the number of progenitor cells which actually have descendants in the adult structure; the latter estimates refer to the total patch area in which the progenitor cells sit. With the present information the most reasonable conclusion is that the progenitor cells for the adult structures are dispersed among other cells which have different developmental fates. If confirmed by experiment, this result has many implications for the process of determination.

INTRODUCTION

It is thought that during oogenesis substances are laid down in particular parts of the egg cortex which determine the fate of cells which derive from that region of the egg. The degree of fineness of that prespecification is unknown. For *Drosophila* it is known that by the blastoderm stage the construction of the adult head or abdomen structures is confined to cells then present in the anterior or posterior halves of the embryo (Chan & Gehring, 1971). By the blastoderm stage

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thoracic precursor cells are also restricted to having their descendants remain in the same segment and possibly in either the anterior or posterior compartment of that segment (Wieschaus & Gehring, 1976*a*; Steiner, 1976).

Except for the special case of the determination of the pole cells (Illmensee & Mahowald, 1974) further restrictions have not been established for the blastoderm stage. Searches for maternal effect mutants which would reveal specific oogenetic determination of organ primordia have so far been unsuccessful (Bakken, 1973; Rice, 1973; Zalokar, Audit & Erk, 1975; Gans, Audit & Masson, 1975).

In this paper we examine published data on genetic mosaics which can bear on this issue. We argue that the cells which are precursors to adult structures are dispersed in the blastoderm among other cells which have different fates. This dispersal makes it unlikely that the imaginal precursors are, at that stage, already determined differently from their intermixed neighbours.

We will examine several methods for estimating the number n of cells in the *Drosophila* embryo which comprise the progenitor populations for various adult structures. Each of the methods involves analysis of mosaic flies in which each individual fly is composed of cells of two different genotypes. This introduction will review published estimates of n based on two methods of inducing such mosaics. Method I uses X-ray induced somatic recombination and method II uses gynandromorphs. These methods base their values for n on the fraction of an adult structure which has descended from a single cell of minority genotype. Both methods yield values compatible with a rather small number of progenitor cells at the blastoderm stage. The Results section will derive two more methods for estimating n using published gynandromorph data. Both methods III and IV base their values for n on the probability of mosaicism occurring within a particular adult structure. Methods III and IV both yield similar estimates, but these values for n at the blastoderm stage are several times larger than those from methods I and II.

In the discussion we argue that the estimates are different because methods I and II estimate the number of cells which actually have descendants in the adult structure, while methods III and IV estimate the number of cells in the region in which the progenitor cells are dispersed.

Method I

X-rays can be used to induce somatic recombination in single cells of the embryo. The descendants of such a cell form a clone of minority genotype visible against a background of contrasting majority genotype. The size of the average clone of minority genotype which is seen in an adult structure can be expressed as a fraction f of the total structure. The number n of primordial cells for that adult structure which existed at the time of irradiation can then be estimated by the formula:

$$n = 1/f,$$

assuming that each of the primordial cells gave rise to an equal portion of the final structure. This formula must be corrected for several effects. Wieschaus & Gehring

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(1976*a*) point out that n should be multiplied by a factor of 0·5 because the marked clone descends from only one of the two daughters of the irradiated cell. Haynie & Bryant (1977) refine this correction to a factor of 0·65 to take account of the non-synchrony of divisions in the precursor population and of the time in the cell cycle when X-rays may induce mitotic recombination. A compensating correction must be made because some of the primordial cells are killed or prevented from mitosing by the X-rays. Haynie & Bryant (1977) estimate that, at the usual dose of 100 *r*, about 50 % of the cells are affected in this way. To correct for this, n should be multiplied by a factor of 2. This gives us a total correction factor of 1·3 and

$$n = \frac{1 \cdot 3}{f}. \qquad (1)$$

Table 1, column 1, presents the published data calculated according to equation (1).

These results give very low estimates for n, ranging from 7 to 26 progenitor cells for a variety of cuticular structures, when X-irradiation is administered at the blastoderm stage.

Method II

The second method uses gynandromorph mosaics in which a chromosome is lost in the first nuclear division; this yields two nuclei of different genotypes. At any later stage about half the cells of the embryo are of each genotype. Because the plane of the first nuclear division is randomly oriented with respect to the axis of the egg and because little nuclear mixing occurs thereafter (Parks, 1936), the dividing line between the two genetically distinct mosaic halves of the embryo is also randomly oriented. Occasionally this mosaic dividing line will pass through a patch of cells comprising the progenitor population for a particular adult structure, resulting in mosaicism for this structure. Whenever the mosaic dividing line separates just one of the n progenitor cells from the remainder of the progenitor population, the descendants of this single cell will form a clone of minimum size in the adult structure. As in method I, the size of such a clone within a particular structure can be expressed as a fraction f of the total structure. Thus a number of adult mosaics can be examined to find those adult structures which have the smallest patch f_{min} of genetically distinct tissue. Each of these patches is assumed to be a clone derived from a single progenitor cell. The number n of progenitor cells can then be estimated by

$$n = 1/f_{min}, \qquad (2)$$

again assuming that each of the progenitor cells contributed equally to the structure. Published results, using this gynandromorph approach, also lead to very low estimates for n, ranging from 8 to 20 progenitor cells for a variety of cuticular and neural structures (Table 1, column 2).

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Table 1. *Estimates of progenitor cell numbers* (n)

Structure	Reference	Footnote	n from X-ray somatic recombination (1)	n based on minimum patch in gynandromorphs (2)	Greatest frequency separation (3)	n from equation (8) (4)	Frequency of mosaicism (4)	n from equation (7) (6)
Eye/antenna disk								
Ant. alone	24	—	9	9	—	—	0·18	62
	25	(a)	9	—	—	—	—	—
Eye and ant.	34	—	16	—	—	—	—	—
	8	(b)	—	> 13	—	—	0·24	104
Eye alone	17	—	—	—	—	—	0·23	96
	3	(c)	< 18–20	—	—	—	—	—
	1	(c, d)	< 18–20	—	—	—	—	—
Wing disk								
Notum only	30	—	—	8–16	—	—	—	—
	20	(c)	< 8	—	—	—	0·146	43
	31	(e)	—	—	—	—	0·125	33
	35	—	—	—	—	—	0·102	23
	27	—	—	—	0·094	44	0·115	29
	8	—	—	—	—	—	0·12	31
Wing and notum wing only	20	(c)	< 10	—	—	—	0·164	52
	27	—	—	—	0·101	49	0·182	63
	17	—	—	—	—	—	0·16	50
	35	(b)	—	—	0·104	52	> 0·147	> 43
	4	—	14	—	—	—	—	—
	7	(b)	—	12	—	—	—	—
	20	(c)	< 20	—	—	—	0·20	75
	27	(f)	—	47?	0·137	85	0·19	68
	—	(g)	—	—	—	—	0·191	69

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Table. 1 (*cont.*)

Leg disk								
	5	—	26	20	—	—	0·25	112
	34	—	20	—	—	—	—	—
1st leg	35	—	—	—	0·141	90	0·220	89
	17	—	—	—	—	—	0·18	62
2nd leg	35	—	—	—	0·126	73	0·190	68
	17	—	—	—	—	—	0·16	50
3rd leg	35	—	—	—	0·106	54	0·155	47
	17	—	—	—	—	—	0·11	27
Abdominal hemi-tergite								
T1	19	—	—	—	—	—	21	81
	19	—	—	—	0·065	23	13	35
T2–T6	10	—	11	8	—	—	0·08	16
	13	—	14	—	—	—	—	—
	34	—	8	—	—	—	—	—
	17	—	—	—	—	—	0·07	13
	11	—	—	—	—	—	0·03	4
Gential disk	11	—	—	—	—	—	0·21	81
Supra-esophageal ganglion	18	—	—	10	—	—	0·18	62
Esophagus stomadeal valve	—	g	32	—	—	—	0·32	176

(a) For antennapedia antenna.

(b) This estimate was limited by the small number of landmarks scored.

(c) Irradiated in first instar. At blastoderm, number should be smaller. $n = 1/f$, not multiplied by 1·3.

(d) From *Drosophila virilis*.

(e) From *Drosophila simulans*.

(f) The published valve of 0·294 for the frequency of mosaicism was incorrect. The author (Rippol, personal communication) has supplied the correct valve which is 0·19.

(g) Personal communication from A. Ferrus. Date to be published in Ferrus & Garcia-Bellido, and Ferrus & Kankel.

? This number must be too high, since direct cell counts, in histological preparations, of the wing disk in newly hatched larvae show only 38 cells. The 38 histologically counted cells include cells destined to become peripodial, tracheal, nerve and muscle cells (Madhavan & Schneiderman, 1977). If there are one or two cell divisions between blastoderm and hatching then the blastoderm cell number would be in the range of 9–19 cells.

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RESULTS

An alternative approach to analysing gynandromorph mosaics is to consider the frequency with which a particular structure is found to be mosaic. Data regarding the frequency of mosaicism within specific structures has been published in two forms: (1) the frequency with which mosaicism will occur anywhere within the structures, and (2) the frequency with which the two most extreme points on the structures will have different genotypes. The following sections will derive formulas for estimating the relative size of a patch of progenitor cells for any adult structure based on these two measures of the frequency of mosaicism.

Method III

The probability that a mosaic dividing line in a gynandromorph passes through a cluster of progenitor cells increases with the size of the cluster. This allows us to derive a formula which will express the area of the patch as a function of the probability p with which the patch is cut by the mosaic dividing line. We idealize the *Drosophila* egg to be a sphere and the dividing line getween the two genotypes to be a great circle on the sphere. We then ask what is the probability that a randomly placed circle passes through a progenitor patch of a given size. The probability should approximate the frequency with which the structure derived from the progenitor patch is observed to be mosaic. The relationship between patch size and mosaic frequency allows us to estimate patch size from observed mosaic frequency.

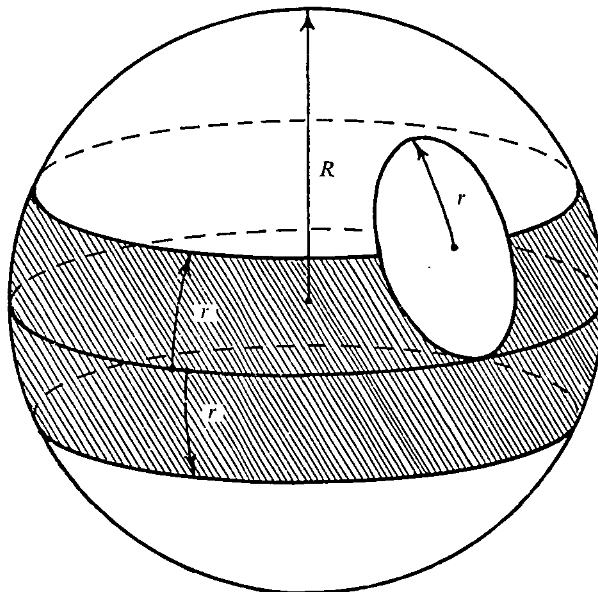

Fig. 1. An idealized spherical blastoderm of radius R. A small circular patch of progenitor cells of radius r will be intersected by the mosaic dividing line (represented by a great circle at the equator) if the centre of the circular patch falls within the shaded zone.

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The probability that a randomly placed great circle passes through a progenitor patch is the same as the probability that a randomly placed patch is cut by a fixed great circle. We assume that the progenitor population is n cells arrayed in a circular patch (see Discussion). The centre of this randomly placed patch may lie anywhere on the sphere with equal probability. The patch is cut by the great circle if the centre of the patch lies above or below the great circle (viewed as an equator) by no more than the distance r (the radius of the patch) as shown in Fig. 1.

The total area in which the centre can lie is then a zone running around the sphere of height r above and below the equator. The area of the zone is then the width of the zone ($2r$) times the circumference of the equator $2\pi R$ (where R is the radius of the sphere), or $4\pi Rr$.

The probability (p) that the centre of the patch lies in this zone is the ratio of the area of the zone to the total area of the sphere ($4\pi R^2$).

$$P = \frac{4\pi Rr}{4\pi R^2} = \frac{r}{R}. \tag{3}$$

These radii can be converted to cell numbers in the following way. Let the surface area occupied by each cell on the blastoderm be one unit. Then the area of a patch of n cells is n, and the total surface area of a blastoderm of N cells is N.

$$n = \pi r^2;\ N = 4\pi R^2,$$
$$r = \sqrt{n/\pi};\ R = \tfrac{1}{2}\sqrt{N/\pi},$$
$$P = \frac{r}{R} = 2\sqrt{n/N}. \tag{4}$$

Rearranging to solve for n,

$$n = \frac{Np^2}{4}. \tag{5}$$

The blastoderm of *Drosophila melanogaster* is now known to contain about 6000 cells (Zalokar & Erk, 1976; Turner & Mahowald, 1976), so

$$n = 1500\,p^2. \tag{6}$$

Note that the equations for zone area and disk area are the plane geometry formulas. This is accurate for small patches, but for large patches spherical geometry formulas must be used. The correct analogs to equations (4) and (5) for a sphere are:

$$p = 2\left[\frac{n}{N} - \frac{n^2}{N^2}\right]^{\frac{1}{2}}$$

or

$$n = \frac{N}{2}(1-\sqrt{1-p^2}).$$

See Nissani & Lipow (1977) for the derivation. The progenitor patch size must be larger than 120 cells before this formula differs by 2 % from formula (5). Thus this correction may be ignored.

In the above derivation, when a progenitor patch is even grazed by the great circle, the resultant patch is considered a mosaic. However in the biological case

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at least one whole cell must be of opposite genotype to create a mosaic. We must thus specify a genotype for single cells on the edge of the patch which are cut by the great circle. Let us assign these cells to the genotype of that part of the blastoderm in which the greater fraction of the cell resides. In this case mosaics will be generated when the centre of the patch lies above or below the great circle by a distance of $(r-\frac{1}{2})$ or less. r is measured in units of cell length, so $\frac{1}{2}$ is $\frac{1}{2}$ of a cell diameter.* When $r-\frac{1}{2}$ is substituted for r in equation (3), equation (6) is corrected to

$$n = 1500p^2 + 68{\cdot}7p + 0{\cdot}08. \tag{7}$$

Although this correction does not materially alter the results, it is not negligible in all cases, so it is used in calculating Table 1.

The number of cells in the progenitor patch for a variety of adult structures has been calculated from equation (7) using experimentally measured values for p that have been reported in the literature. Results are listed in Table 1, columns 5 and 6. The precursor populations estimated in this way are much larger, generally in the range of 50–100 cells. Internal structures also show a very high frequency of mosaicism, examples of which are shown in Table 2.

Table 2

Structure	Reference	Frequency of mosaicism	n from equation (7)
Crop	17	0·11	27
Cardia	17	0·15	45
Anterior midgut	17	0·64	659
Posterior midgut	17	0·29	147
Anterior Malphigian tubules	17	0·37	232
Posterior Malphigian tubules	17	0·40	268
Outer genitalia	17	0·23	96
Inner genitalia	17	0·12	31
Gonadal mesoderm	11	0·37	232
Supra-esophageal ganglion	18	0·18	62

Method IV

Method IV is derived from method III, but allows us to use gynandromorph data reported in a different way. In some papers, the frequency with which particular points on an adult structure are separated by the mosaic dividing line is reported. Hotta & Benzer (1973) give a formula which relates the frequency of separation of the two farthest cells (d) to the total frequency of mosaicism for a circular patch (p):

$$p = \pi d/2.$$

* This same $r-\frac{1}{2}$ applies if one assumes that any arbitrary fraction (q) of the cell must reside above the equator for the cell to be of that genotype. A mosaic is created if the patch lies no more than $r-q$ below the equator or $r-(1-q)$ above the equator. The total zone width is then the sum of these two or $2r-1$ which is the same as $2\,(r-\frac{1}{2})$.

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Wieschaus & Gehring (1976*b*) calculated the frequency of separation between the two most widely separated pair of bristles for the wing and leg disks, and also measured the total frequency of mosaicism within these disks. They found that the maximum divergence from this formula was 10 %. They argue that since this formula fits their data very well, the disk primordia can best be regarded as circular.

If $\pi d/2$ is substituted for p in equation (7), then equation (8) results.

$$n = 3700\, d^2 + 108\, d + 0{\cdot}8. \tag{8}$$

Using equation (8) data reported in terms of d can be used to estimate the number of cells in the patch. These results are shown in the third and fourth column of Table 1. The estimates are again large, generally ranging from 44 to 90 cells.

DISCUSSION

This paper has described four methods for estimating a number associated with an adult structure which we have called n, the number of progenitor cells for that structure. Note that the values for n from methods I and II are similar, and that the values from methods III and IV are also similar. However, the estimates based on the latter two methods are several times greater than those based on the former two methods.

A recent report by Nissani & Lipow (1977) also compares estimates for progenitor populations based on method I and a frequency-of-mosaicism calculation similar to our method III. Their conclusion, that the two methods yield similar values, is not supported by their data: they cite only six frequency-of-mosaicism measurements. Two of their estimates clearly reveal the discrepancy we emphasize in this paper. These authors also use an obsolete value of 3400 (Sonnenblick, 1950) for the number of cells on the blastoderm surface. If a more accurate estimate of 6000 blastoderm cells (see Zalokar & Erk, 1976; Turner & Mahowald, 1976) is used in their calculation, the discrepancy appears in every item in their data table.

We must now consider just what these numbers are measuring, and whether the discrepancy among the different values can be meaningfully interpreted. We shall discuss the possibility that (A) the assumptions for some of the methods may lead to bias in the results; and (B) the estimates may not refer to the same populations of cells.

(A) *Systematic bias*

Method I. Analysis of somatic recombination mosaics depends on the assumption that the marked cell and the other cells of the progenitor population each contributed a similar number of descendants to the final structure. This assumption is not true; different cells of an imaginal disk can divide at quite different rates (Garcia-Bellido & Merriam, 1971). Nonetheless if each of the progenitor cells has an equal probability of being induced to crossover by X-rays, the *average* descendant clone size will reflect the number of progenitors at the time of irradiation.

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Damaged cells may give rise to smaller than normal clones; there is however no reason to expect that cells showing crossover events, having been struck by X-irradiation, would be less damaged than other cells. Hence the average clone size descending from X-ray hit cells would reasonably be expected to be smaller, but not larger, than normal. Such an error would cause an inflated value for n, causing the values in Table 1 to be overestimated. On the other hand, if the X-irradiation kills some of the progenitor cells, the remaining cells will be estimated, leading to an underestimate for n. A correction to account for this last effect has been included in equation (1).

Method II. Like method I discussed above, method II also assumes progenitor cells contributed equally to the adult structure. As mentioned above it is known that the different cells of an imaginal disk can divide at different rates (Garcia-Bellido & Merriam, 1971*a*). Since a slowly proliferating cell would give rise to a smaller than average clone, the descendants of such a cell would be scored as the minimal patch size, resulting in an inflated value for the estimated number of progenitor cells. Similarly, since this gynandromorph method is only likely to mark single cells if these cells occur at the edge of a cluster of progenitor cells, and since such edge cells may also contribute descendants to adjacent structures (Wieschaus & Gehring, 1976*a*), such cells may be expected to give rise to a smaller than average number of descendants and hence be responsible for a minimal patch of marked tissue. Again, this would tend to cause the value for n to be an overestimate.

Hence most systematic errors in methods I and II, lead to overestimation rather than underestimation. Correcting the biases inherent in these methods would probably enhance the discrepancy of these estimates from those of methods III and IV.

Methods III and IV. The data base for these two methods is the frequency of mosaicism within a structure or between two points on a structure. The measured frequencies are rather large (all greater than 12 %). Failure to notice some marginal cases of mosaicism (method III) would lead to a measured frequency lower than actual and hence to a lower than actual estimate for n. Method IV should also slightly underestimate the number of progenitor cells, since the two farthest scorable cells in the adult may not derive from the two farthest separated progenitor cells.

The assumptions of the derivations (both use identical assumptions) refer to an idealized model that is clearly different from biological reality: (1) The egg is a sphere; (2) the two mosaic halves are randomly oriented hemispheres. A third assumption, that the progenitor cells are arrayed in a circular patch, has been discussed above.

1. The assumption that the egg is a sphere causes little trouble. Adaptation of the derivation for a more realistic ellipsoid would slightly reduce the probability of mosaicism for structures derived from polar locations on the egg while structures derived from middle-locations would show a correspondingly increased probability. The experimental evidence indicates that mosaic probability for both polar structures (eye, antenna) and medial structures (wing, legs) is significantly greater

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than would be expected from the progenitor cell numbers based on methods I and II.

2. The assumption that the mosaic border divides the egg randomly into two equal halves is supported by experimental evidence: in a series of gynandromorphs any structure has about a 50–50 chance of being of male or female genotype (Garcia-Bellido & Merriam, 1969; Kankel & Hall, 1976; Ripoll, 1972; Wieschaus & Gehring, 1976*b*).

A more difficult question is whether the border between the two halves approximates a smooth line. A highly convoluted border line would lead to a higher frequency of mosaicism. To explain the data discussed here the line would have to be so convoluted that every patch of 10–20 cells would have a 10–25 % probability of being cut by the line. Hotta & Benzer (1973) argue that 'the fact that a self-consistent map can be constructed indicates that such mixing [leading to a jagged border] is not prohibitively large...where the boundary passes through the eye [the irregularities of the boundary] usually do not exceed a few ommatidial diameters'. We have no further evidence on this question; a convoluted border presents a reasonable alternative to the hypothesis we favour.

Thus it seems that the biases in the four methods (except for the possibility of a convoluted border) are either insufficient or acting in the wrong direction to explain the discrepancies between the estimates for progenitor cell numbers.

(B) *Referent populations*

We now consider the possibility that the estimates may not refer to exactly the same set of cells.

Methods I and II estimate the number of cells which actually have descendants in the adult tissue. Methods III and IV estimate the area of the patch in which the progenitor cells lie. Methods III and IV do not require or assume that all the cells in the patch have descendants in the adult structure. These methods only depend on the distances between the progenitor cells. If a small number of progenitor cells were dispersed in a larger patch, then methods I and II would refer to the small number of progenitor cells, and methods III and IV would refer to the total number of cells in the patch. We propose this as the most likely explanation for the discrepancy. A small number of progenitor cells dispersed in a larger patch would have a high probability of being separated by a gynandromorph dividing line. This seems to be the most reasonable way in which the 15 or so progenitor cells for the eye/antenna disk could show an 18–24 % frequency of mosaicism (Table 1).

The intervening cells could make larval tissue, could make unscored internal adult tissue, or could die before adulthood. Note that *all* the descendants of any interspersed cell must die, or lie in other tissues, since the interspersed cells contribute *no* progeny to the adult structure in question. Thus the possibility that 'a single blastoderm cell might give rise to both larval and imaginal structures' (Wieschaus & Gehring, 1976*b*) cannot explain the present data. On the other hand the possibility that 'a given area of the blastoderm might contain [some] cells of larval [prospective significance] and [other cells of] imaginal prospective sig-

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nificance' (Wieschaus & Gehring, 1976*b*) would explain the data. This last possibility has been suggested several times on various grounds (Sturtevant, 1929; Geigy, 1931; Garcia-Bellido & Merriam, 1969; Wieschaus & Gehring, 1976*b*). We believe that our analysis of the data at hand supports this hypothesis.

Conclusion

At the blastoderm stage there are a limited number of precursor cells which develop into each adult structure. These are the precursor cells that have been counted by the somatic recombination studies and by the gynandromorph studies enumerated in Table 1, columns 1 and 2. We conclude that these few precursor cells are dispersed among a considerably larger cluster of cells which do not contribute to these adult structures.

There is evidence that the cells in the blastoderm are already at least partly determined and that the determination depends on the position the cells occupy in the blastoderm (Chan & Gehring, 1971). It is simplest to presume that *all* the cells which occupy a particular small patch of the egg cortex become fixed in the same developmental fate. The data analysed in this paper would argue that this is not the case. We suggest that either (1) at the time of determination cells which acquire one fate are interspersed with cells that acquire another fate, or (2) a particular step of determination does not occur until the cells have rearranged themselves so that cells of the same fate are adjacent. In the latter case, it is possible that imaginal disks are not formed from a continuous group of cells which invaginates, but by inwandering of cells scattered over an area. Determination as to a larval or an adult fate may not occur until the cells migrate inward to form imaginal disks.

The method for derivation of equation 3 was originally suggested to us by Professor Harold Morowitz. Subsequently we noticed similar derivations in Wieschaus (1974) and in Nissani & Lipow (1977).

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